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BOVINE BRUCELLOSIS.

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THE discovery of Dr. Bernhard Bang of the germ of epizootic abortion in the womb of a cow in 1895 was an event of great importance in the history of animal pathology, for it speedily put a stop to speculation regarding the cause of the disease, and served as the starting point of researches which extended knowledge and corrected many erroneous beliefs.

Since the disease had henceforth to be enrolled with the contagious maladies, the question of diagnosis became of the first importance, and it was natural to consider the possibility of employing some of the methods that had already been used with success in contagious diseases of the human subject.

The general belief at this date was that epizootic abortion was a disease of the cow, that infection was spread mainly or exclusively by the bull, and no thought was given to the possibility that there might be other methods of infection or that bovine animals at any age and of either sex might be susceptible to infection.

Immediately following Bang's discovery of the causal germ of the disease the examination of the uterine contents, fœtus, and discharges from the womb, for the detection of the bacteria became a practicable method of diagnosis, and it was applied by McFadyean and Stockman as early as 1909, the material being obtained from the owners of infected herds, who were asked to forward to the laboratory aborted calves and membranes for examination. Sterilised cotton-wool swabs fixed on wire were forwarded to owners, who were asked to insert them into the genital passages of animals that had recently aborted and return them to the laboratory for examination, to ascertain the value of that method for diagnosis in the event of no calf or membranes being available. By the examination of such materials it was possible to confirm the existence of epizootic abortion on 55 farms distributed over 36 counties in England.

It marked a considerable advance to be able to make a positive diagnosis in this way when materials were actually obtainable for examination from the diseased cow or the aborted fœtus, but there still remained to be discovered a reliable method of diagnosing the disease in affected animals before abortion had taken place. This part of the problem was investigated along three different lines : (1) By the agglutination test ; (2) by the fixation of the complement method ; (3) by a method similar to the use of tuberculin and mallein, with *abortin*—a material prepared from cultures of the abortion bacillus.

We are here concerned only with the bare statement of the methods of diagnosis that were experimentally employed, but it is important to note that the methods of employing the agglutination and complement tests were very fully described, and actually corresponded with those which are in use throughout the world at the present day, in medical as well as in veterinary practice. What may be termed the theory as well as the practice of these methods of diagnosis was also fully described.

It was of course realised that in order to be able to employ the agglutinin test for diagnosis one must first know how normal animals reacted to it.

It seemed obvious that the best method of determining this was to test a large series of samples taken at random from young non-pregnant heifers of from bulls or steers.* Blood was accordingly obtained and tested from over 500 animals of that kind. The age of the heifers and steers varied from $1\frac{1}{2}$ to $3\frac{1}{2}$ years and that of the bulls from 2 to 5 or 6 years. In the great majority of these cases the blood was taken while the animals were being killed in the normal course of business at the slaughter-house in London, and their history was not obtainable.

A detailed account was published of all these cases and the summary was as follows :—

Altogether the serum from 535 presumably healthy cattle (485 steers, 34 bulls, 6 heifers, and 10 calves) was tested, and in 526 of these the serum when tested with dilutions containing not less than 1 : 50 failed to cause any distinct agglutination.

In nine animals some agglutination occurred with a dilution of 1 : 50. Details regarding the reaction in these nine cases were shown in regard to tubes containing 1 : 50, 1 : 100, and 1 : 200.

Details of the tests of about 200 other cases were given at the same time.

Finally, in an interpretation of the results, it was said that the agglutination test had now passed the probationary stage and deserved to be adopted as the method of diagnosis most generally applicable in suspected cases of contagious abortion. It was stated that the discovery of such a reliable method of diagnosis removed what had hitherto been the greatest obstacle in grappling successfully with the disease

* In accordance with the current belief that the disease attacked cows only.

either by private effort or under State regulations. It was also pointed out that it provided an easy means of ascertaining whether there was any real foundation for the view that other organisms than the abortion bacillus discovered by Bang were responsible for abortion on an epizootic scale.

The Agglutination Test.

The emulsion or suspension of *Br. abortus* employed by McFadyean and Stockman was first adopted arbitrarily, but an endeavour was made to keep it constant by reserving from each brew one or more samples in small tubes of the same calibre. These were sealed and kept at room temperature to serve as a guide in the dilution of the next brew, and so on. There were abundant opportunities to renew the samples of both normal and infected blood.

Other workers at the same period also generally adopted arbitrary standards, and consequently it was impossible to institute an exact comparison of the results obtained and published by different authors.

Precision in describing the composition of the mixture of bacteria and serum in each tube of a series was greatly facilitated by the researches of Brown (*Ind. Jl. Med. Res.*, 1915, Vol. II, p. 763), and of Cunningham and Timothy (*Ind. Jl. Med. Res.*, 1924, p. 1253). The former constructed a series of ten tubes, numbered 1 to 10, graduated according to the amount of precipitate of barium sulphate which each contained, and by using the hæmocytometer method Cunningham and Timothy were able to calculate the bacterial contents of Brown's tubes for different organisms.

By this method Dr. Leslie Sheather in 1928 determined the titre of the standard emulsion which had been continually employed at the Royal Veterinary College. It was found to correspond to Brown's No. 3 tube and contained 3·3 million of the *Br. abortus* per c.mm. (This calculation was made on the assumption that the figure was the same as the one which had been determined by Cunningham and Timothy for the *Br. melitensis*).

Nearly all the published standard suspensions used in testing for contagious abortion are much weaker, that is they contain fewer bacteria, but it may be inferred that the one employed in Sweden by Henriccson was similar to the one used in London up to 1928, as the critical tube was 1 : 10.

A very extensive use of the system which regards distinct agglutination in the 1 : 10 tube (corresponding to Brown's No. 3) as evidence of infection has proved entirely trustworthy, but it is perhaps inferior to some other systems for determining the ultimate titre ; that is, the minimum amount of serum that will produce a recognisable positive reaction.

It must be mentioned, however, that other factors than the relative proportion of serum and bacteria in the suspension affect the result—notably the temperature at which the test is carried out and the period at which the result is read off, which it would be out of place to discuss here.

Students and practitioners should be warned that there is no safe or reliable test for contagious abortion for anyone without experience and the resources of a laboratory, and this advice applies with greater force in regard to employing the complement test.

The Value of Single Tests.

Successive agglutination or complement tests of a bovine animal's blood serum with a titre above normal (1 : 10) often show that the titre gradually declines and ultimately becomes negative—a result which is generally accepted as evidence of recovery, meaning thereby disappearance of the *Br. abortus* from the system. It follows that when the first test of an animal shows a titre of 1 : 10 that is not proof that the animal is not infected, for it is also consistent with the view that infection has only recently taken place.

Consideration of this fact emphasises the importance of employing tests which include a tube such as the 1 : 10, in which a strong positive result may be accepted as evidence that the animal may be the subject of active disease, with living bacilli in its body, and is not in the convalescent stage.

In fact, however, it is only a warning sign and does not prove infection, because it is conceivable that small amounts of agglutinin may persist in the blood for some days after the disappearance of the last bacterium.

The proper action in such a case is to suspend judgment, have the animal isolated, and retest its blood after a month.

A point that is often overlooked in connection with the agglutination test is that even a negative result of the 1 : 10 test does not justify the verdict " not infected." That is obvious, because in cases of natural infection by the digestive or genital tract there is no reason to expect that on the day of the first test the amount of agglutinin in the blood will be sufficient to produce a positive result in 1 : 10. The accurate interpretation of the result of a first test is possible only when it is positive at 1 : 10. That is because it has been proved that the serum of normal bovine animals is negative at that titre.

It must also be for a time the titre of many naturally infected cattle, for both clinical observation and experiment have shown that susceptibility to the agglutination test, has what may be called a stage of incubation, which may sometimes extend to weeks when infection has occurred naturally with a small amount of brucella bacteria taken in by the mouth. The analogy here is with tuberculous infection and the time that must be allowed to elapse before expecting a reaction to the tuberculin test.

Some authors seem to like to record very high reactions, up to the terminal point, such as 1 : 1,000, but in eradication tests, these have no importance compared with missing an infected animal because the series of tubes used did not include a 1 : 10.

In every infected animal there is a day on which there would be a positive reaction in a 1 : 10 tube and not in any higher dilution.

Practical Application of the Agglutination Test in Dealing with Affected Herds.

By the year 1911 such confidence in the test had been gained that the time appeared to be ripe for its employment in an effort to eradicate the disease from definite herds. Through the Journal of the Royal Agricultural Society of England and in other ways this was made public, and many applications for the proffered assistance were received. Complete tests of many herds were made, and the increasing experience only served to confirm the extraordinarily trustworthy character of the test.

It was, however, soon realised that eradication of the disease in this way was in many herds impracticable from the outset, not because of any failures of the test, but because of the large proportion of cows proved by the test to be already infected and the fact that in many cases the owner of the herd could not afford to adopt the course which was held to be necessary, *viz.*, to put out of his herd every animal that had reacted to the agglutination test. That was, and still is, the state of affairs in the majority of milking herds.

Fortunately no difficulty was encountered in obtaining offers of herds in which this difficulty did not occur. These were pedigree herds in which complete and permanent separation between the diseased and healthy animals could be maintained either on separate farms or on completely separate parts of the same farm.

It was felt that the success of such a plan would fully establish the reliability of the test and encourage the use of this method for dealing with the disease.

Fully detailed accounts of the steps by which herds had been freed from the disease and the reliability of the test thereby established were published in this Journal, and it ceased to be necessary to discuss a question generally regarded as solved. It is re-opened here because evidence that completely erroneous views regarding the diagnosis and prevention of the disease are now being spread.

Eradication.

In a Report published in 1921 it was stated that the method employed, which relied upon the agglutination test for the detection of the disease, had been entirely successful, but that in the majority of the outbreaks in which the advice and assistance of the Institute had been sought the plan was seen to be impracticable from the outset or had to be abandoned after a trial.

It would be of little interest now to describe in detail the history of infected herds in which the possibility of eradication was never put to the test. These were herds in which inspection and preliminary tests showed that the conditions necessary for eradication were absent. These conditions were (1) only a moderate rate of

infection of the herd, (2) not merely the consent but the whole-hearted interest and co-operation of the owner and his foreman in the work, and (3) a possibility of effecting complete separation between the infected and the non-infected members of the herd as determined by the agglutination test.

Herd No. 1.

This is a herd in which the existence of contagious abortion was first determined in 1911 by agglutination tests carried out in the case of cows that had actually aborted, and early in that year it was suggested to the owner that it might be worth while to attempt to eradicate by carrying out repeated tests at short intervals and making arrangements for the isolation of the animals thus found to be infected.

This advice was not taken at that time, and in the latter part of 1911 the young heifers were inoculated before service. This was not repeated in the following years, and cases of abortion continued to occur at intervals among the cows in spite of the usual disinfection and temporary isolation.

In 1914 the owner was able for the first time to make provision for isolating animals found to be infected, and he then agreed to put into operation the system of eradication previously suggested. The first general test of the herd was accordingly begun in April of that year.

Three tubes were used for the test of each blood, *viz* :—

½ tube = 1 of serum to 50 of bacterial emulsion.
1 „ = 1 „ 100 „ „
2 „ = 1 „ 200 „ „

In some of the later tests a ¼ tube with 1 of serum to 25 of emulsion was used.

To distinguish different degrees of agglutination in these tubes the terms used were : comp. = complete, adv. = advanced, dist. = distinct, apprec. = appreciable, and neg. = no agglutination.

Lot 1. Test No 1. April 23rd, 1914.

In this first lot of 36 animals all were cows or young heifers in calf.

In the record each animal was assigned a number from 1 to 36, but in the list they were also designated by their names. The result of the test was positive in the following cases :—

No. 5. Aggl. complete in all the tubes. This was one of the five in-calf heifers.

No. 7. All comp. Suspected in 1911 but the result of a test was then negative.

No. 15. All comp.

No. 23. All comp.

No. 29. ½ comp., 1 nearly comp., 2 dist. Abortion in 1908.

No. 30. All comp. Aborted in March, 1913, and then reacted to the test.

No. 34. All comp. Aborted in 1908.

In the case of the remaining 30 animals in this lot the result of the test was completely negative.

Points of interest in connection with animals in which the test was negative were :—

Nos. 9, 19 and 20 were cows that had been inoculated with live vaccine in 1914.

No. 25 had recently calved, six days prematurely.

No. 27. Although negative in this test failed in the next on June 29th.

One of the heifers that did not react was No. 4 J——a, and about a fortnight after this test she calved a weakly calf which the owner thought to be a few days too soon, and as she had been in contact with No. 5, which the test showed to be infected, another sample of blood was taken from her on May 13th. This second test, like the first, had an entirely negative result.

Lot 2. *Test No.* 1. *April 27th,* 1914.

BLOOD FROM 13 ANIMALS, 37 TO 50.

No. 37. ½ comp., 1 nearly comp., 2 dist. Never known to abort but a test in 1911 was positive.

No. 39. ½ apprec., 1 and 2 neg.

No. 47. All comp. Was herself a premature calf and had aborted in July, 1913.

No. 48. ½ apprec., 1 and 2 neg. Inoculated October, 1911. (Tested again, with negative result, on July 6th, 1914.)

No. 50. All comp. Aborted in 1904 and 1905.

Result negative in all the others.

In this lot cows in which the test was completely negative although they had been inoculated in 1911 were Nos. 38, 40, 43 and 49.

Lot 3. *Test No.* 1. *April 30th,* 1914.

Blood from 36 animals, 51 to 87, were included in this lot. The results were as follows :—

No. 52. All comp.

No. 56. All comp. Not known to have aborted but had previously been tested in November, 1912, with the result : ½ adv., 1 dist., 2 neg.

No. 58. ½ dist., 1 and 2 neg. Aborted 1907. Tested in April, 1912 : ½ comp., 1 adv., 2 neg. Now 12 years old. (Tested again with negative result on July 6th and November 17th, 1914.)

No. 61. ½ apprec., 1 and 2 neg. Inoculated December, 1911. (Was negative when next retested November 22nd, 1915.)

No. 74. ½ comp., 1 dist., 2 neg. Calved at full time, December, 1911, but showed slight reaction to the test afterwards.

No. 75. All comp. Had an apparently healthy calf, nine days prematurely on August 22nd, 1912. Her blood was tested on the following day, result : ½ and 1 comp., 2 adv.

No. 76. ½ and 1 comp., 2 nearly comp. Aborted 1907.

No. 84. All comp. Was inoculated December, 1911.

No. 87. All comp.

In this lot of animals in addition to No. 61 there were four others that had been inoculated in August, 1911, but did not now react to the test, *viz* : 68, 81, 82 and 84.

No. 52 above had a calf born on March 20th, 1914, and its blood when tested on January 4th, 1915, was quite negative.

Lot 4. *Test No.* 1. *May* 4*th*, 1914.

This lot included 34 animals and in the following six the result was positive.

No. 98. All comp. Had been in the herd many years and had a calf every year but one.

No. 99. All comp. Aborted March, 1911.

No. 100. All comp. Aborted 1908.

No. 104. All comp. Aborted August, 1913.

No. 105. ½ and 1 comp., 2 dist.

No. 114. All comp.

Points of interest in connection with animals negative to the test in this lot were :—

No. 94. Failed in a later test, March 29th, 1915.

No. 102. Failed when retested on August 25th.

No. 103. Failed when retested on June 29th.

No. 107. Had been inoculated in 1911 and had first calf at full time in 1913. Had twins at full time in March, 1914, and a blood test immediately afterwards was negative.

Lot 5. *Test No.* 1. *May* 7*th*, 1914.

This lot included 18 heifers, five of which were dairy heifers in calf, and the others pedigree heifers about to be served.

The result of the test was negative in all these animals.

HERD 1. SECOND TEST OF COWS AND FIRST TEST OF YOUNG HEIFERS.

June 29th, 1914.—On this date blood was received from 37 animals previously tested with a negative result. The test was again negative, except in two :—

No. 27. All comp. Passed the previous test but had since aborted.

No. 103. ½ comp., 1 nearly comp., 2 neg. Was negative to test on May 4th.

July 6*th*, 1914.—Blood was forwarded for a second test from 37 other animals and the result was negative in every case. This lot included one cow (No. 48) which was inoculated in October, 1911, and showed appreciable agglutination in the ½ tube in the test on April 28th, 1914.

July 9*th*, 1914.—Thirty-one samples of blood were tested for the second time, all with negative result.

July 16*th*, 1914.—Blood was received from three cows that were negative to the first test. The result was again negative in each case. The owner reported that these were the last of the breeding animals which passed the first test.

July 29th, 1914.—Blood was forwarded for the first time from eleven yearling heifers—142 to 152 inclusive, and tested with negative result in each case. One of these (No. 148) failed when afterwards tested on December 3rd.

No. 150. Had sucked and ran with a cow which had aborted.

No. 151 was a premature calf now about a year old, which ran with and sucked its dam till quite recently.

August 3rd, 1914.—On this date blood was tested with negative results from 22 animals that had passed their second test on June 29th. It was thought advisable to have an early retest of these animals because they had been in contact with No. 27 and No. 103, which had been found to be infected on June 29th. This lot included No. 66, which afterwards failed on October 27th.

August 3rd, 1914.—Blood also tested with negative result from a young in-calf heifer, No. 153.

August 25th, 1914.—Blood was forwarded from the following two animals :—

No. 154. Result negative.

No. 102. ½ and 1 comp., 2 nearly comp.

This last animal, now found to be infected, had passed three previous tests, *viz.*, on May 4th, June 29th and August 3rd, but had calved prematurely since August 4th and she had formed part of the lot in which two reactors were discovered in the test on June 29th.

The fact that this animal calved prematurely within three weeks after a test of her blood had proved negative naturally tended to disturb the owner's confidence in the reliability of the test, and to some extent our own. It was considered probable that the animal had been infected shortly before the test on June 29th or immediately after that date, before No. 103 and No. 27 had been isolated.

September 21st, 1914.—Blood was sent from No. 13, Roan Irish, and No. 10, Roan Little, and the result of the test was negative in both cases. This was the fourth negative test of these two animals, as they were included in the 22 from which blood was sent on August 3rd (see above).

September 28th, 1914.—Blood was forwarded from the following two animals, and tested with negative result :—

No. 11. Red R——n.

No. 112. Roan P——r.

Both of these animals had calved since the previous test and, according to calculation, two or three days prematurely. It was thought well to have their blood retested immediately on that account, and also because one of them had only recently been going with five others that were known to be infected.

At this date the owner explained that after each previous test he had as far as was possible immediately removed from the herd the animals found to be infected, but in the above case and a few others that could not be arranged.

October 27th, 1914.—Blood was forwarded from 20 animals, all of which had previously been tested with negative result. On this occasion the result was again negative except in two, in which it was as follows :—

No. 66. Carol. ½ and 1 comp., 2 nearly comp.
No. 70. Violet. ½ and 1 comp., 2 nearly comp.

In a letter subsequently received the owner attributed the infection of these two animals to contact with the cow No. 102, (test on August 25th, 1914), which animal calved prematurely in the field in which Carol and Violet were at the same time grazing.

Both Carol and Violet had been tested with negative result on April 30th, June 29th and August 3rd.

Another General Test.

It was decided to carry out another test of all the cows and heifers that had been previously tested with negative results, and the animals were accordingly tested as follows :—

November 17th, 1914.—Twenty animals tested, all with negative results.

November 22nd, 1914.—Thirty-two animals tested, all with negative results. The lot included No. 61, which was inoculated in December, 1911, and showed appreciable agglutination in the ½ tube when tested on April 27th, 1914.

November 30th, 1914.—Thirty animals tested, all with negative results.

December 3rd, 1914.—On this date ten samples of blood were tested with completely negative results in all cases except one. The exceptional animal was No. 148, in which the test had the following result : ½ dist., 1 and 2 neg.

This heifer, now pregnant, had been tested with negative result before service on July 27th.

December 21st, 1914.—A sample of blood was forwarded from one of the cows tested with negative result on November 30th, and the test on this occasion was also negative. It was subsequently learned from the owner that the cow (Roan crossbred) had aborted about three months before time, and that the case appeared to be one of typical contagious abortion. The expulsion of the fœtus was quite sudden, and a quantity of unhealthy-looking discharge subsequently came away. It was thought that the calf had been dead for some time before expulsion.

Until he received the report that the test of the blood was negative the owner had been greatly perturbed because there appeared to have been no opportunity for the cow's infection during the previous seven or eight months and she had passed all previous tests.

Although it was thought that the case was very probably not one of contagious abortion it was recommended that the cow should be isolated and that a second sample of blood should be forwarded from her after a month (see test on February 3rd, 1915).

January 4th, 1915.—On this date blood was forwarded from the following six animals, and the result of the test was negative in each case :—

No. 116. Polled No. 7.
No. 118. Polled No. 9.
No. 71. Polled No. 2.
No. 45. Rosebud.
No. 128. Polled No. 13.
No. 17. Small Ears.

Blood had previously been tested with negative result from all these cows. No. 17 had aborted a few days before the blood was sent, and it was reported that the calf was only about half the size corresponding with the period of pregnancy.

February 3rd, 1915.—On this date blood was forwarded from the following three animals and tested with negative results :—

Roan cross-bred cow. Previously tested with negative result on December 1st, 1914.

No. 17. Small Ears. Previously tested with negative result on January 4th, 1915.

No. 96. Isabel. This cow was tested again as an extra precaution because the owner had been obliged to keep her at the same buildings as the reacting cows for some time after the last previous test.

February 6th, 1915.—On this date blood was forwarded from the following two animals and tested with entirely negative results :—

No. 108. Red and White. Recently calved about 15 days prematurely and did not cleanse properly.

No. 82. Jess. Was reported to have calved a few days before the expected date, but there was no other ground for suspicion.

February 25th, 1915.—On this date a further sample of blood was forwarded from each of the above two cows, and the test was again entirely negative.

March 29th, 1915. On this date blood was forwarded from the heifer, No. 94, L. R. 6th, and a test had the following result : $\frac{1}{2}$ dist., 1 apprec., 2 neg.

It was reported that the blood was sent because the heifer had been showing indications of impending abortion, and after the test had been carried out the owner was informed that the cow should be considered infected, but it was recommended that another sample of blood should be forwarded after a month. It was afterwards learned that she aborted on March 31st, at the seventh month, and as she was not considered of great value she was sold to a butcher without waiting for the result of another test of her blood.

April 8th, 1915.—On this date blood was forwarded from No. 148, Saucy. This was a two-year-old heifer whose blood had been previously tested on December 3rd, 1914, on which occasion there was agglutination only in the $\frac{1}{2}$ tube (1 in 50). The result of the present test was negative.

It was subsequently ascertained that the heifer was not due to calve till May 12th, but that on April 6th she had given birth to a calf that lived for a few hours only. She was sold to a butcher soon afterwards, and unfortunately, therefore, her blood was not tested again.

June 8th, 1915.—On this date blood was forwarded from No. 142, Flower, and No. 149, Aura, and tested with completely negative result.

Blood was sent from these two animals because they had been in close contact with the heifer No. 148, mentioned above, which aborted on April 6th, and also because No. 142 had given birth to a rather weakly calf, which was thought to be a little too soon and lived only a few days.

September 24th, 1915.—On this date blood was forwarded from the following four animals, and tested with completely negative result in each case :—

No. 14. Blue Grey. Blood was sent from this cow because she had been in contact with one or two of the animals that were found to be infected by the agglutination test, and the owner now wished to transfer her to the healthy section of the herd.

Princess P. } These two were young heifer calves that had been sucking
Sc. Butterfly } reacting cows.

Dairy Crossbred R. Blood was sent from this animal because she had calved the previous day, and this was thought to be a few days too soon. There was no other ground for suspicion.

Bella.—Blood was sent from this cow on November 22nd, 1915 with completely negative result. The cow had been newly purchased for introduction into the healthy herd. She had previously had two calves full term.

Third General Test.

December 15th, 1915.—It was decided to carry out another general test of the herd, and accordingly on this date blood was forwarded from 19 cows and 10 heifers. The result of the test was entirely negative in each of these cases.

On January 4th, 1916, another batch of blood was forwarded from 30 cows and heifers. The result of the test was negative in every case.

On January 12th, 1916, blood was forwarded from another lot of 28 cows and heifers, and the result was again negative in every case.

On January 17th, 1916, blood was forwarded from 26 animals, and again tested with completely negative results.

On February 5th, 1916, the general test of the herd was completed by testing blood from four bulls. The result of the test was quite negative in these cases also.

The above was the last general test of the herd. After it had been carried out the owner was advised that if any cases of abortion or premature birth occurred in any animal in the healthy portion of the

herd blood should without fail be forwarded to the laboratory. This advice was followed, and the particulars with regard to these cases are given below. It may be noted here, however, that the last case of new infection in the herd was the one detected on March 29th, 1915, *viz*, L. R. 6th.

In letter dated June 25th, 1920, the owner reported that the last of the reacting cows, which had been isolated on a separate farm, were sold the previous autumn, and that the herd included no animal that had ever reacted to the agglutination test except four heifers which had reacted temporarily as calves.

Agglutination Titre in Cases in which the First General Test had a Positive Result.

In the spring of 1914, when the attempt to eradicate the disease was first begun, the herd comprised 109 cows that had had one or more calves, 18 heifers in calf or recently calved, and 29 younger heifers. (The young calves and the bulls in the herd at that time were not tested.)

On the occasion of the first test there was at least appreciable agglutination in 25 cases, not including four animals which had been inoculated as heifers in 1914.

In 18 of the 25 cases agglutination was complete up to 1 in 200, and in nine of these (Nos. 5, 7, 15, 23, 52, 56, 87, 98 and 114) there was no history of abortion. The other nine had aborted or calved prematurely as follows :—

M. Butterfly. Aborted March, 1914.
No. 30. Aborted March, 1913.
No. 34. Aborted 1908.
No. 47. Aborted July, 1913.
No. 50. Aborted 1904 and 1905.
No. 75. Calved prematurely in 1912.
No. 99. Aborted 1911.
No. 100. Aborted 1908.
No. 104. Aborted 1913.

The remaining seven cases, with a lower agglutination titre, were :—

No. 29. ½ comp., 1 nearly comp., 2 dist. Aborted 1908.
No. 37. ½ comp., 1 nearly comp., 2 dist. Never aborted.
No. 39. ½ apprec., others neg. Never aborted.
No. 58. ½ dist., others neg. Aborted 1907.
No. 74. ½ comp., 1 dist., 2 neg. Never aborted.
No. 76. ½ and 1 comp., 2 nearly comp. Aborted 1907.
No. 105. ½ and 1 comp., 2 dist. Never aborted.

The foregoing cases in which the titre of the serum was up to (and may have exceeded) 1 in 200 for complete agglutination in cows that had previously aborted are of great interest, because such a titre suggests either the long persistence of the disease in them or reinfection, the former being the more probable. Further evidence of long-

continuing disease will be found in the subsequent history of Nos. 52, 56, 75 and 87.

Other facts of special interest are (1) that the first test had a positive result in every animal with a history of previous abortion, and (2) that it had a negative result in all the young heifers that had not yet been served.

Herd No. 2.

The first sample from this herd was forwarded on September 18th, 1917, and the test had a positive result, the agglutination being complete in 1 : 200. The owner was informed that the result showed that the cow in question (afterwards known as No. 9) had been infected with contagious abortion and it was then learned that she had been purchased in the previous March and on August 24th had calved six weeks before the proper time. The calf was born alive but died almost immediately.

The last previous owner, when informed of the abortion, affirmed that the cow could not have been infected while in his possession, but the present owner held the contrary opinion, as he had no reason to suspect its previous existence in his herd at any time during the past five years.

Immediately after the abortion the cow had been placed in a shed by herself, and the cows which had been in the same field were put into a fresh field and strictly isolated from the rest of the herd, which at this time numbered about 130 head, divided into two lots kept on different premises—South Farm and North Farm. The animals that had been in the same field with the infected cow at this time were the following :—

No. 2. Due to calve January 30th, 1918.
No. 3. Due to calve January 7th, 1918.
No. 4. Due to calve February 12th, 1918.
Nos. 5 and 6. Twin heifers not supposed to be in calf.
No. 7. Due to calve January 14th, 1918. Her previous calf had been born 3½ weeks prematurely but survived and did well.
No. 8. Due to calve December 6th, 1917.
No. 13. A bull calf sucking cow No. 9.

Blood from these animals and also from No. 9 (second test) was taken on October 23rd, 1917, and when tested the result was positive in two of them, No. 9 and No. 4. In the former case agglutination was again complete in all tubes up to 1 : 200, and in No. 4 agglutination was advanced in 1 : 50 and negative in the higher dilutions.

On the same date blood was tested from a cow, No. 12, which had been purchased at the same time and from the same owner as No. 9, and from two cows, Nos. 10 and 11, which had been kept with No. 12. Also from a cow No. 1 which had never been near No. 9, but in February, 1917, had calved a dead calf at 8 months. The result of the test was negative in all these cases.

In December the owner intimated that No. 4, which was not due to calve till February 12th, had aborted on December 5th, a fact which he considered strong evidence of the accuracy of the test.

On December 12th, 1917, blood was again forwarded from Nos. 2, 3, 5, 6, 7 and 8, with negative result in all of them except No. 2, in which case agglutination was advanced in ¼ tube (1 : 25) but negative in the others, and the owner was advised to have this animal strictly isolated for a month and then to send another sample of blood from her.

On receipt of this advice the owner removed Nos. 3, 5, 6, 7 and 8 to a fresh field and left No. 2 where she had been since the first case of abortion on August 24th.

On January 16th the owner intimated that No. 2, which was not due till the 30th of the month, had a dead calf on the 5th and that No. 7, which was due on January 14th had calved on December 2nd ; the calf was weakly and did not survive.

In the meantime No. 3 and No. 8 had calved live calves at full time.

On January 23rd, 1918, blood was again sent from Nos. 2, 3, 5, 6, 7 and 8, and also from another cow, No. 14, which had calved a little too early. This cow formed part of the herd that had never been in contact with the infected lot. The result of the test was as follows :—

No. 2. Aggl. complete in all tubes up to 1 : 200.
No. 3. All neg.
No. 5. All neg.
No. 6. ¼ adv., ½ dist., 1 and 2 neg.
No. 7. Comp. in ¼, ½ and 1, nearly comp. in 2.
No. 8. All neg.
No. 14. All neg.

The interesting facts in connection with these tests are :—

(1) The increase in the agglutination titre of No. 2 since the first test on December 12th, and

(2) The failure of Nos. 6 and 7 which had both passed the previous test.

As already noted, No. 7 had calved prematurely in the interval between the two tests.

No further communication was received regarding the herd until March 27th, 1918, when the owner intimated that No. 6 had aborted on January 31st previous.

At this time, No. 9, the cow that first aborted on the farm, *viz*, on August 24th, together with the bull calf (No. 13) sucking her, and Nos. 2, 4, 5, 6 and 7 were kept together but isolated from the remainder of the herd.

No. 3, which had passed all the previous tests, and had been kept separate with her live full-term calf since December 24th, and No. 8, which had passed all the tests and now had a live calf, had been separated from the infected lot on November 30th.

The owner was informed that no good purpose would be served by having another test of the reacting animals at this time ; but as he was anxious to return Nos. 3 and 8 to the healthy herd, if that was considered safe, blood was forwarded from these two animals on May 15th, and also from two new animals, Nos. 15 and 16. No. 15 was a cow which the owner thought was either carrying a dead calf or was about to calve prematurely, and No. 16 had had a dead calf taken from her on May 4th. She was not due to calve till May 29th. The result of the test was as follows :—

No. 3. ¼ adv., others neg.
No. 8. All neg.
No. 15. All comp. (Up to 1 : 200.)
No. 16. All neg.

A further test was made of the serum of No. 15 in order to see what was the height of the agglutination titre and it was found to be nearly complete in 1 : 500, and distinct in 1 : 1,000.

When these results were intimated to the owner it was learned from him that Nos. 15 and 16 had not been in contact with any of the animals previously suspected or found to be infected. They belonged to that part of the herd which was kept at South Farm into which no animal had been brought from outside for less than about two years. Moreover, as previously mentioned, no cases of abortion except those previously noted had occurred in any other part of the herd during the previous five years. The animals isolated in consequence of the case of abortion on August 24th, 1917, had been attended to by a man who never came near the rest of the farm and the place where these animals were kept was a mile distant from the rest of the herd.

On May 28th blood was again forwarded from the cows Nos. 15 and 16 and from another cow, No. 17, which also belonged to the herd at South Farm.

The test had an entirely negative result in Nos. 16 and 17 and in No. 15 agglutination was again complete in 1 : 200, the highest dilution used.

The owner was advised to have blood tested from all the animals at South Farm, and the first lot, from 36 animals, was taken on June 12th. The test had a negative result in all the cases except the following :—

No. 28. Comp. in all tubes (up to 1 : 200).
No. 29. Dist. in ¼ tube (1 : 25) only.
No. 31. Comp. in all tubes.

The owner was informed that Nos. 28 and 31 were certainly infected and that No. 29 should be considered doubtful. It was recommended that No. 29 should be isolated from both diseased and healthy animals and that another sample of blood should be sent from her in a fortnight.

A letter subsequently received from the owner conveyed the information that Nos. 28, 29 and 31 were non-pedigree dairy cows which had been purchased as heifers three years previously, along with another, No. 30, blood from which had been included in the recent test.

No. 28 had had three calves since purchased, her last being born on May 22nd, 1918, nine days before the expected time. The calf was alive when born but survived only five hours.

The other reacting animal, No. 31, was pregnant and due to calve on August 22nd next, and it was subsequently learned that she calved on that very day. Her previous calf had been carried to full term.

The animal considered doubtful as the result of the test, No. 29, had had a healthy calf at full term on March 19th previous.

With the exception of the above-mentioned four dairy cows all the animals included in the test on June 12th were pedigree animals kept at South Farm, and since purchase the dairy cows had not been in any way isolated from the other animals kept there.

The owner now reported that he had three dairy cows which had also been purchased as heifers in 1915 at the same time and place as the above-mentioned four dairy cows and that they were in part of the herd at North Farm.

On September 17th, 1918, blood was forwarded from 39 animals at South Farm, these including all those that had not reacted to the previous test, and also No. 29 in which the result of the previous test was doubtful.

On this occasion the test of No. 29 was quite negative, but there were two new reactors, Nos. 19 and 30.

No. 19. $\frac{1}{4}$ comp., $\frac{1}{2}$ nearly comp. It was afterwards ascertained that this animal was a pedigree bull and that blood from him had passed the previous test on June 12th.

No. 30. $\frac{1}{4}$ and $\frac{1}{2}$ tubes both comp. This, it should be noticed, was one of the non-pedigree dairy cows which had been purchased three years previously, and had passed the test on June 12th. Her last calf had been born at full term on April 22nd, and she had been served again on July 31st by a yearling bull which was afterwards sent to the butcher.

At the same date, September 17th, blood was sent from the following six animals belonging to the North Farm portion of the herd :—

Nos. 52, 53 and 54. These were the cross-bred dairy cows previously referred to, which had been purchased three years ago. Nos. 55, 56 and 57. These were three pedigree cows which had not been breeding and which the owner thought ought therefore to be tested. He had, however, no other reason to suppose that abortion existed in this part of the herd.

The only reactor in this lot of six animals was the crossbred No. 53, in which agglutination was complete in both the $\frac{1}{4}$ and $\frac{1}{2}$ tubes. She had calved a full-time healthy calf on June 22nd and had not since been served. She was immediately isolated.

As the owner at this time was not quite satisfied with the results of the recent test, a general test of all the other animals at North Farm was not immediately carried out, but on November 20th blood was again sent from the above crossbred cows at that farm and tested with the same negative result in Nos. 52 and 54, and with a positive result in No. 53 : $\frac{1}{4}$ comp., $\frac{1}{2}$ nearly comp.

In view of this confirmation of the previous test of No. 53, the owner now decided to have all the remaining animals at North Farm tested, and blood was forwarded from them (numbered 60 to 94 inclusive) on November 30th, 1918. The result was negative in all of them.

On May 28th, 1919, blood was forwarded from 25 of the animals at South Farm (Nos. 22 to 46), all of which had been tested with a negative result on June 15th and September 20th, 1918. On this occasion the result was again quite negative except in the following two :—

No. 22. $\frac{1}{4}$ and $\frac{1}{2}$ comp. This cow had given birth to a healthy calf at full term on January 11th, 1919, and on April 27th following had been served by a bull (No. 20) which passed the present test. She had not settled to this service.

No. 46. $\frac{1}{4}$ and $\frac{1}{2}$ tubes both dist. This was a heifer which had been served for the first time on January 25th, 1919, by the same bull as No. 22. These were the only animals served by this bull that ever reacted.

As the owner was not quite satisfied with regard to the result of the above test, blood was again forwarded from Nos. 22 and 46 on June 4th and the test had the same result as before. These two animals were then removed from the healthy herd and isolated, and it was suggested that another test of the non-reacting animals at South Farm should be carried out after a month or six weeks. Blood was accordingly sent from them (35 in number) on July 23rd, 1919, and the result of the test was quite negative in every case.

Up to the time of writing (May, 1921) no case of abortion had occurred in the herd at either of the two farms.

Subsequent History of the Infected Animals.

A number of the infected animals were put out of the herd soon after their infection was detected, but the following, on account of their value, were retained for a time in isolation, in the hope that it might eventually be possible to return them with safety to the herd, or at least to put their progeny into the herd.

Cow No. 2.

October 20th, 1917. Tested for the first time, with negative result.
December 12th, 1917. Tested : $\frac{1}{4}$ adv., $\frac{1}{2}$ neg.
January 5th, 1918. Had a dead calf three weeks premature.
January 23rd, 1918. Tested : Comp. up to 1 : 200.
May 29th, 1919. Tested : Comp. in 1 : 50 (highest dilution used).
January 15th, 1920. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

The above dead calf was to a service by bull No. 19 on April 25th, 1917, but a test of the bull's blood on June 12th had a negative result.

Cow No. 4.

October 23rd, 1917. Tested for the first time. Result : ¼ nearly comp., ½ adv., 1 neg. Due to calve February 12th.
December 5th, 1917. Aborted.
September 4th, 1919. Gave birth at full term to a bull calf, No. 107.
January 15th, 1920. Tested : ¼ nearly comp., ½ adv.
November 26th, 1920. Tested : ¼ nearly comp., ½ adv.

Calf No. 107.

September 19th, 1919. Tested when 15 days old : ¼ nearly comp., ½ very adv.
November 26th, 1920. Tested : Result negative.

The owner was advised that he might regard the animal as free from infection.

Heifer No. 5.

October 23rd, 1917. Tested for the first time. Result negative.
December 12th, 1917, and January 23rd, 1918. Tested with a negative result on both occasions.
May 28th, 1919. Tested : ¼ adv., ½ dist.
June 27th, 1919. Gave birth at full time to a dead calf (breech presentation). This was her first calf.
January 15th, 1920. Tested : ¼ dist., ½ neg.

Heifer No. 6.

October 23rd and December 12th, 1917. Tested with negative result.
January 23rd, 1918. Tested : ¼ adv., ½ dist., 1 neg.
January 31st, 1918. Aborted. Date of last service August 13th, 1917.
May 28th, 1919. Tested : ¼ and ½ comp.
August 26th, 1919. Gave birth to a full-term calf, No. 106.
January 15th, 1920. Tested : ¼ and ½ comp.
November 26th, 1920. Tested : ¼ comp., ½ nearly comp.

Calf No. 106.

January 15th, 1920. Tested for the first time when five months old. Result negative.

Cow No. 7.

October 23rd, 1917. Tested for the first time, with negative result.
December 12th, 1917. Again tested with the same result.
December 22nd, 1917. Aborted. Date of last service, April 9th, 1917.
January 23rd, 1918. Tested : ¼, ½ and 1 comp., 2 nearly comp.
May 28th, 1918. Tested : ¼ comp., ½ nearly comp.
January 15th, 1920. Tested : ¼ comp., ½ dist.
March 21st, 1920. Gave birth to a full-term calf, No. 110.
November 26th, 1920. Tested : ¼ apprec., ½ neg.

Calf No. 110.

November 26th, 1920. Tested for the first time, with negative result, when eight months old.

Cow No. 9.

A cow which had been purchased by the present owner in March, 1917, with the following history. She had a live calf on January 14th, 1916, and

was not served again until September 20th following. She was again served on November 26th and January 6th following, and she calved about six weeks prematurely on August 24th, 1917. The calf was born alive but died almost immediately.

September 18th, 1917. Tested for the first time. Result positive up to 1 : 200.

October 20th, 1917. Again tested with the same result.

February 18th, 1919. Gave birth to a full-term calf.

May 26th, 1919. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

January 19th, 1920. Tested : $\frac{1}{4}$ comp., $\frac{1}{2}$ dist.

July 3rd, 1920. Gave birth to a full-term calf, No. 112.

November 26th, 1920. Tested : $\frac{1}{4}$ nearly comp., $\frac{1}{2}$ adv.

Calf No. 112.

November 26th, 1920. Tested when nearly five months old, with negative result.

Cow No. 15.

May 13th, 1918. Tested for the first time : $\frac{1}{4}$ and $\frac{1}{2}$ comp., and nearly comp. in 1 : 500. The cow was pregnant, and due to calve on July 27th.

November 22nd, 1918. Tested : comp. in 1 : 50 (highest dilution used).

February 7th, 1919. Gave birth to a decayed fœtus.

May 28th, 1919. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

January 15th, 1920. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

June 1st, 1920. Produced a full-term calf.

November 26th, 1920. Tested : $\frac{1}{4}$ nearly comp., $\frac{1}{2}$ dist.

Cow No. 30.

April 22nd, 1918. Calved at full term.

June 12th, 1918. Tested for the first time, with negative result.

July 31st, 1918. Served by a yearling bull out of a cow that afterwards passed the test.

September 17th, 1918. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ both comp.

November 22nd, 1918. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ nearly comp.

May 9th, 1919. Gave birth to a full-term calf.

May 28th, 1919. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ nearly comp.

November 26th, 1920. Tested : $\frac{1}{4}$ comp., $\frac{1}{2}$ nearly comp.

Cow No. 22.

This cow was tested with a negative result on June 15th and September 20th, 1918. She had a full-term calf on January 11th, 1919, and when tested on May 26th, 1919, the result was positive : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

November 26th, 1920. Tested : $\frac{1}{4}$ nearly comp., $\frac{1}{2}$ adv.

This cow fostered the following calf.

Calf No. 108.

This was a bull calf born on February 15th, 1919, out of a tested cow (No. 34), and it was fostered by the above cow (No. 22).

The calf was tested on three occasions, *viz.*, September 19th, 1919, January 19th, 1920, and July 26th, 1920, with a negative result on each occasion.

Bull No. 19.

This animal was born in another herd on March 13th, 1915, and purchased by the present owner in March, 1916.

June 12th, 1918. Tested for the first time, with negative result.

September 17th, 1918. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

November 22nd, 1918. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ nearly comp.

May 28th, 1919. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

January 15th, 1920. Tested : $\frac{1}{4}$ and $\frac{1}{2}$ comp.

In 1917 this bull served the cows Nos. 2, 4 and 6, the last on August 13th. These three cows afterwards became infected, presumably through contact with the cow that aborted in August, 1917, as the bull's blood was negative to a test on June 12th, 1918. All the cows served by the bull between August 13th, 1917, and September, 1918, either passed the test before or after service. He was not used for service after his infection was detected in September, 1918, and he was sold for slaughter in 1920.

Bull No. 95.

This was a young bull which was being used exclusively for the service of the infected cows during their isolation. He had been put with these animals before he was tested for the first time on May 28th, 1919, and agglutination was then advanced in the 1 : 25 tube and distinct in 1 : 50.

Blood from the bull was again tested on September 23rd, 1919, and agglutination was then only appreciable in 1 : 25 and negative in 1 : 50.

Summary.

The owner of this herd was strongly of opinion that his cattle had been free from contagious abortion for several years, and that the disease was introduced by a cow which was purchased in March and aborted in August, 1917, but the subsequent blood tests revealed the existence of infection in cows that had been in the herd for some years and had never been in association with the suspected cow. Having regard to all the facts, it appears to be probable that the disease had been slumbering in the herd for an indefinite time, but that the cow purchased in March, 1917, brought a fresh source of infection. However that may be, it can hardly be doubted that the system of testing and isolation saved the owner from what might have been a serious outbreak and eventually eradicated the disease. How much saving this meant can be inferred when it is stated that every live calf born in this herd was valued at £100.

As in the case of the herd previously dealt with, the repeated tests indicated that the bulls had nothing to do with the spread of the disease, since the first positive result had no apparent relationship to the date of service. They also showed the long persistence of the disease in some of the animals, including one of the bulls, and the rapid decline of the agglutination in calves out of infected cows.

Herd No. 3.

In this herd repeated tests were carried out at intervals during a period of ten years, but no proper segregation of the affected animals could be arranged and the owner was reluctant to make the sacrifice

in putting them out of the herd immediately. It was a valuable herd and during the last ten years of its existence it had been almost entirely self-recruited, although occasionally new bulls or heifers were introduced by purchase. It was excellently managed and the sanitary conditions were good, although the premises did not permit of complete isolation of diseased or suspected animals, either in the houses or at pasture.

At various times after 1918 the advisability of resorting to inoculation with live vaccine prepared by the Ministry of Agriculture was considered, but it was not advised, as it was thought better to continue the efforts to eradicate the disease without that.

History of the Herd.

The herd first came under observation in 1913, at which time it included 35 cows, one or two bulls, and about 20 heifers and calves.

Blood from three cows was tested in January of that year, and the result was negative in No. 3 but positive as follows in Nos. 1 and 2 :—

No. 1. ½ and 1 comp., 2 nearly comp.
No. 2. ½, 1 and 2 comp.

No history of the cows was obtained at this time, but the owner was informed that these two animals had been infected with contagious abortion.

Blood was again tested from No. 1 on June 7th, 1913, and the result was exactly the same as in January. The cow's history which was then obtained was as follows :—

Her first calf was born in 1910 at full term, and in 1911 the calf was three weeks before time, and in each of the following two years parturition was again premature to about the same extent.

On August 14th, 1913, blood was sent from three other cows because they had unexpectedly turned to the bull, but the result of the test in each case was negative.

On April 22nd, 1914, blood was tested with positive result from a heifer (No. 3) which had had a dead calf about a month previously. The exact date of service was not known but the owner had thought that the abortion was the result of an accident.

Immediately after this the owner was strongly advised to have a general blood test of the herd carried out, and that was begun on May 11th.

First General Test, May 11th, 1914.

The herd at this time comprised 27 cows and 18 heifers, of which only seven had been served. The result of the test was positive in the following eight cases :—

No. 4. ½, 1 and 2 comp.

It was learned that this cow had calved normally in 1905, but it was a long time afterwards before she stood to service and her second calf was born at full term in May, 1907. She calved normally in each of the following

six years and was due to calve on May 22nd. She had twin calves in 1912 and both died soon after birth. The owner suspected that the cow might have aborted between her calf in 1905 and the one in 1907.

No. 5. ½, 1 and 2 all comp.

This cow was five years old and had calves normally in 1911 and the two following years. She was due to calve again about the middle of June.

No. 1. All tubes comp.

Blood from this cow had been tested on two previous occasions, the last being on January 20th, 1913 (see above). She was now in calf to a service on September 29th, 1913.

No. 6. All tubes comp.

This cow was six years old and had had calves in each of the years 1909-1913, the first and last of these being premature. She was now in calf again.

No. 7. Result positive in all the tubes.

The cow was 13 years old and had had ten calves, the first in 1904 and the last in 1914. The first calf was born at the eighth month but it survived and did well. In the years 1906 and 1911 there had been difficulty in getting her settled to the bull.

No. 8. ½ comp., 1 and 2 neg.

The animal was born in September, 1912, and was now carrying her first calf, the date of service having been February 8th, 1914.

No. 9. ½, 1 and 2 all comp.

This was a heifer born in October, 1912, and now supposed to be in calf to a service on March 27th, 1914.

No. 10. ½, 1 and 2 all comp.

This was a heifer born in July, 1913, and not yet served. The dam of this heifer was No. 5 above.

It was now arranged that to prevent the further spread of the disease the reacting animals should be isolated as far as practicable, and that those that were not of special value should be put out of the herd.

Second General Test, October 27th, 1914.

At this time the herd comprised 55 animals, of which 43 were cows or heifers in calf, two were bulls, and the remaining ten were young heifers that had not yet been served.

The test had a positive result in eight animals, of which the following four had reacted in the previous test above :—

No. 5. ½ comp., 1 nearly comp., 2 adv.

Since the previous test the cow had calved 12 or 14 days prematurely on June 2nd, but the calf was alive and apparently normal. Since that date she had been served three times and the owner had decided to put her out of the herd.

No. 4. ½ nearly comp., 1 adv., 2 apprec.

This was a less positive result than the one in the preceding test in the month of May. Since the previous test she had calved normally on May 23rd.

No. 7. Comp. in all three tubes.

This was the same result as in the previous test.

No. 10. ½ and 1 comp., 2 nearly comp.

This heifer (which had never been served) had previously reacted (see above) ; it was now put out of the herd.

The following were four new reactors :—

No. 11. All three tubes comp.

This cow was ten years old. She had had nine calves, the first in 1906 and the last in March, 1914. All of them had been born at full time.

No. 12. ½ comp., 1 and 2 adv.

This animal was born in February, 1912, and first served on July 10th, 1913. The calf was born prematurely on March 22nd, 1914. She had since been served twice, the last date being October 14th. (For some unexplained reason this animal's blood had not been included in the first general test.)

No. 13. All three tubes comp.

A cow born in April, 1910, and had had three calves, the last being born on June 16th, 1914. She had since been served twice.

No. 14. ¼ nearly comp., 1 apprec., 2 neg.

This was a bull born on July 24th, 1912. It was sold immediately after the test.

In the remaining 47 animals included in this test the result was completely negative.

Third General Test.

In April, 1915, it was ascertained that the herd still comprised five of the animals that had previously been found to be infected, and four that had lately aborted, and the owner was strongly advised to have another general test carried out. The advice was accepted and the test was carried out on April 27th. On this occasion blood was sent from 49 animals, and the result of the test was positive in the following seven, none of which had reacted to the previous test :—

No. 15. D.A.IV.—All three tubes comp. (½, 1 and 2).

The cow was born on December 18th, 1911, and had had two calves, the first born at full term on January 14th, 1914, and the second born a month prematurely on November 24th, 1914. She had been served again on March 15th, 1915, but the owner intended to put her out of the herd immediately after this test.

No. 16. O.A.II.—All three tubes comp.

This cow was seven years old. She had full-term calves in 1910, 1911, 1913, and 1914, and twins prematurely in 1912. She had since been served on February 16th, 1915, and was apparently in calf. She had passed the two previous general tests.

No. 17. N.A.II.—All three tubes comp.

This was a five-year-old cow, and she had had four calves born at full term, the last on November 23rd, 1914. Since the last parturition she had been served three times, apparently without success, and the owner decided to put her out of the herd.

No. 18. S.D.—All three tubes comp.

This animal was born on May 5th, 1913, and she had a calf about two months prematurely in April, 1915. She was an animal of great value.

No. 19. D.A.III.—All three tubes comp.

This animal was born in November, 1911, and she had had a full-term calf in 1913 and 1914. The latter was born on November 21st, 1914, and she had since been served on January 26th, 1915.

No. 20. L.R.—All three tubes comp.

This was an eight-year-old cow, and she had had a full-term calf in each year since 1910, the last having been born on March 6th, 1915. She had not since been served, and it was intended to get rid of her.

No. 21. C.51.—All three tubes comp.

This animal was bought in May, 1914, at which time she was 14 months old. She had been served before purchase and had a full-term calf on June 25th, 1914. She was next served on September 5th, 1914, and she calved prematurely on March 24th, 1915. She was considered a very valuable animal, and the owner was reluctant to put her out of the herd.

Fourth General Test.

The fourth general test was not carried out until May 10th, 1916. On this occasion blood was sent from 50 animals, and in eleven of these the result was positive, six being new reactors.

The following five had been found to be infected in a previous test, and the result of the present test in each of them was as follows :—

No. 7. S.R.—½ comp., 1 apprec., 2 neg.

This old cow had been barren since the previous test in October, 1914.

No. 12. D.R.V.—The result of the test was distinct in ½ and negative in 1 and 2.

At the time of the test the cow was pregnant and due to calve on August 9th, 1916.

No. 13. N.D.—Comp. in all three tubes.

Now pregnant and due to calve June 10th, 1916.

No. 15. D.A.IV.—Comp. in all three tubes.

She had calved at full term in December, 1915, and was again pregnant.

No. 18. S.D.—½ comp., 1 adv., and 2 neg.

She had a full-term calf a week after this test (1916).

The following are the six animals in which the result of the test was positive for the first time :—

No. 22. S.A.—Comp. in all three tubes.

Born February 14th, 1914, served June 5th, 1915, and calved prematurely February 14th, 1916.

No. 23. S.A.V.—Comp. in all three tubes.

Born January, 1914, served July 5th, 1915, and calved prematurely on April 1st, 1916. The calf was alive, but died immediately.

No. 24. E.C.—Comp. in all three tubes.

Born February 24th, 1913. She had a calf at normal time in 1915, was served on June 4th, 1915, and calved prematurely on January 11th, 1916.

No. 25. S.A.—Comp. in all three tubes.

Born August 5th, 1913, and had a normal calf in 1915. She was afterwards served on October 23rd, and calved prematurely on April 10th, 1916.

No. 26. O.R.II.—Comp. in all three tubes.

Born February, 1912, and calved prematurely in March, 1914. The second calf was carried to full term, and she was now pregnant and due to calve in August, 1916.

No. 27. S.R.—½ and 1 comp., 2 nearly comp.

Born in December, 1913, served August 25th, 1915, and calved prematurely April 27th, 1916.

After this test the owner decided to put four of the above cows out of the herd, *viz.*, Nos. 7, 25, 26 and 27, but on account of their value he was reluctant to part with the others.

After the test it was reported that since the preceding one the affected animals retained in the herd had been isolated as far as possible, and it was decided to continue that course.

Nothing further was herd about the herd until the end of December, 1917, when it was reported that there had been three fresh cases of abortion during the previous two months. The owner therefore suggested another general test of the herd, and that was carried out on January 1st, 1918.

Fifth General Test.

The number of animals from which blood was sent was 54, and the result of the test was negative except in the following five cases, of which three had previously reacted and two were new infections. The previous reactors were as follows :—

No. 15. D.A.IV.—¼* dist., ½ adv., 1 and 2 nearly comp., but inversely graded. (Paradoxal reaction.)

Since the previous test the cow was served on March 12th, 1917, and had a dead calf on December 2nd, 1917.

No. 22. S.A.—¼ and ½ nearly comp., 1 apprec., 2 neg.

This cow had a full-term calf on March 7th, 1917, and had been served afterwards on June 19th.

No. 23. S.A.V.—¼ comp., ½ nearly comp., 1 apprec., and 2 neg.

The cow had a calf at normal time on April 7th, 1917, and was served again on June 18th following.

The following were two new reactors :—

No. 28. H.A.II.—¼, ½ and 1 comp., 2 nearly comp.

Born December 17th, 1914, and had a normal calf on January 17th, 1917. Served again on March 15th, 1917, and aborted on October 26th.

No. 29. H.R.II.—¼, ½ and 1 comp., 2 nearly comp.

Born in January, 1915, served May 3rd, 1916, and calved February 12th, 1917. The calf died. She was again served on April 28th, 1917, and calved prematurely on December 29th following.

In spite of continued attempts to prevent the spread of the disease by isolating the infected animals as far as possible, it was reported at the end of September, 1918, that there had been three recent cases of abortion in the herd.

It was therefore decided to have another general test, and that was carried out on October 14th, 1918.

Sixth General Test.

On this occasion blood was sent from 48 animals, of which six were bulls, and the others cows and heifers. In all, the test indicated seven infected animals, but only three of these were new reactors. The previous reactors were as follows :—

No. 15. D.A.IV.—¼ and ½ nearly comp.

This cow had not had a calf since the last previous test.

No. 22. S.A.—¼ nearly comp., ½ adv.

* It will be observed that a ¼ tube (1 : 25) was now included in the series.

Since the last previous test the cow had a normal calf on March 31st, 1918.

No. 23. S.A.V.—$\frac{1}{2}$ nearly comp., $\frac{1}{2}$ adv.

Since the previous test the cow had a calf at normal time in April, 1918. On this occasion the only tubes used were 1 : 25 ($\frac{1}{4}$ tube) and 1 : 50 ($\frac{1}{2}$ tube).

No. 28. H.A.II.—$\frac{1}{4}$ and $\frac{1}{2}$ comp.

The cow had been served on March 6th, 1918.

The following are the three cases of new infection :—

No. 30. S.S.R.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both nearly comp.

This animal had had a normal calf in 1915, 1916 and 1917, but the fourth calf was premature on September 29th, 1918.

No. 31. S.A.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

This was a heifer whose first service was in November, 1917, and she calved prematurely on June 16th, 1918.

No. 32. P.A.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

Born March, 1917, and now supposed to be in calf to a service on June 16th, 1918.

Included in this lot of animals there were two which had not reacted although it was reported that they had aborted or calved prematurely. The first of these, S.S.R., had been served on January 18th, 1918, and calved September 12th, 1918. The other was S.F. She had her first calf at full term in December, 1917, after which she was served on February 19th, 1918, and calved prematurely on September 28th, 1918.

Seventh General Test.

The next general test was carried out on May 12th, 1919. The total number of animals tested was 46, and the result was positive in eight cases, of which five were animals that had previously reacted and three were new infections.

The animals previously infected were :—

No. 28. H.A.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

The last previous test was in January, 1918. She had been served on March 6th and calved on December 15th, 1918. The calf was very weakly and died.

No. 30. S.S.R.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp. She had not been served since she reacted to the previous test in October, 1918.

No. 31. S.A.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

At present pregnant and due to calve September 8th.,

No. 32. P.A.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

Since the last test she calved prematurely on January 16th, 1919.

No. 22. S.A.—$\frac{1}{4}$ comp., $\frac{1}{2}$ nearly comp.

Reacted to three previous tests, the first in May, 1916. Her last calf was born March 31st, 1918, and she had since been barren.

The following three animals reacted for the first time :—

No. 33. H.R.V.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

She had three calves all at full time, and the fourth was born prematurely on February 24th, 1919, to a service on July 24th, 1918.

No. 34. P.A.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

This was a heifer born November, 1917, and her first service was on April 15th, 1919.

No. 35. P.A.III.—¼ and ½ both nearly comp., graded.

This was a young calf, born on March 22nd, 1919, out of No. 23. S.A.V. (See Fourth General Test on May 10th, 1916.) It was the fourth calf out of that cow, and only the first was premature (269 days).

Eighth General Test.

This was carried out on June 16th, 1920. The number of animals tested on this occasion was 43. With the exception of one bull all the animals were cows and heifers. The result of the test was positive in five, but the following four had previously been found to be infected :—

No. 32. P.A.—¼ and ½ both comp.
No. 28. H.A.II.—¼ and ½ both comp.
No. 33. H.R.V.—¼ and ½ both comp.
Had a full-term calf on April 20th, 1920.
No. 31. S.A.II.—¼ and ½ both comp.

The fifth animal, which now reacted for the first time, was :—

No. 36. P.R.II., in which the result of the test was ¼ nearly comp., ½ dist.

This animal had her first calf on January 14th, 1920, and she had not since been served.

Ninth General Test.

This was carried out on February 13th, 1921. The total number of animals tested was 56, and with the exception of one bull all the animals were cows or heifers. The result of the test was positive in the following four cases :—

No. 36. P.R.II.—¼ and ½ both comp.

This animal reacted to the previous test, and since then she had been served on four occasions, the last being on October 19th, 1920. Note the increased titre.

No. 33. H.R.V.—¼ and ½ both comp.

Reacted to the two previous tests. She had not been served since her last calf and was now nearly dry.

No. 31. S.A.II.—¼ and ½ both nearly comp.

A reactor since October, 1918. She had bred regularly since then.

No. 37. C.A.—¼ and ½ both comp.

This was a new reactor. She was a heifer and had been first served on October 8th, 1920, and afterwards on four occasions, the last being January 5th.

It was advised that all these four reacting animals should be got rid of, in the hope that the disease might thereby be eradicated.

On March 1st, 1921, it was reported that No. 35, P.A.III, had aborted on February 27th. This animal had been born on March 22nd, 1919, and her blood was tested in May, 1919, with positive result. Her dam was the reacting cow No. 23, S.A.V. Blood was forwarded from No. 35 on March 5th, and the result of the test was negative.

On June 5th, 1921, blood was sent from four animals, and in the following two the test had a positive result :—

No. 38. P.C.—$\frac{1}{4}$ very adv., $\frac{1}{2}$ apprec.
No. 39. P.C.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.

In the other two cases the test had a negative result.

All of these animals had passed the last previous test, and each had calved in the preceding month, but three of the calves were very weak at birth and died soon afterwards. The pregnancy lasted 278 days in No. 38 and 262 days in No. 39. It was suspected that the cows had been injured or frightened, because they were grazing in a meadow which, without the owner's knowledge, was used for three or four weeks for the training of steeplechase horses. The owner was advised that Nos. 38 and 39 must be considered infected, and that another general test should be carried out immediately. It was, however, delayed until January 13th, 1922.

Tenth General Test.

On this occasion blood was sent from 48 animals. Twenty-three of them were cows in calf or in milk, ten were heifers in calf, two were bulls, and the remaining thirteen were young heifers that had not been served. The result of the test indicated that only three of the animals were infected. These were as follows :—

No. 38. P.C.—$\frac{1}{4}$ comp., $\frac{1}{2}$ adv.
Previous reactor. She had had three calves, and was again in calf.
No. 39. P.C.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.
Previous reactor. Now carrying her third calf.
No. 40. C.A.II.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.
This was a new infection. It was now ascertained that this cow had aborted her first calf in August, 1921.

The owner was urged to make every effort to isolate the three remaining infected animals if it was not possible to put them out of the herd immediately.

Eleventh General Test.

This was carried out on December 14th, 1922, the herd then comprising 43 animals. The result was more or less positive in the following five animals :—

No. 41. C.C.—Agglutination complete up to 1 in 500.
This cow had passed the previous two tests, but she had calved her second calf prematurely on March 2nd, 1922, and was not now in calf.
No. 40. C.A.II.—$\frac{1}{4}$ comp., $\frac{1}{2}$ nearly comp.
Reacted to previous test. She had her first calf prematurely in August, 1921, and her second in August, 1922. She was afterwards served on October 20th, 1922.
No. 38. P.C.—$\frac{1}{4}$ and $\frac{1}{2}$ both comp.
Previous reactor, but always calved at full time. She was at present carrying a calf.

No. 39. P.C.II.—¼ and ½ both comp, 1 nearly comp., 2 neg.
Previous reactor. It was learned that she had calved prematurely shortly before the test.
No. 42. P.A.II.—¼ dist., ½ neg.
This was a heifer that had been served for the first time five weeks before the test.

The last test of any animal in the herd was made on July 18th, 1923, when blood was sent again from the cow No. 38, P.C., which had reacted to the two previous tests. The result of the test was ¼ and ½ both comp.

It was understood that this was the last reacting animal left in the herd, and it was sold to a purchaser who was previously informed regarding her history.

Discussion.

The preceding history is of some interest as showing the insidious way in which contagious abortion continues to spread in a herd when diseased and healthy animals have to be kept on the same premises, and it is not a little remarkable that in the ten years no less than 40 cows or heifers and one bull contracted the disease. This is without reckoning the calf No. 35 which was no doubt born infected. The majority, if not the whole of the other calves out of infected cows, were probably also infected at birth, but No. 35 was the only one from which blood was tested so soon.

A point of particular interest is the detection of the disease in the ten-months-old heifer No. 10. The dam was found to be infected at the same test, but it is not certain that she was already diseased when this calf was born. Assuming that she was infected at that time, the calf's blood would no doubt have given a positive result, but it is already established by many observations that in cases of congenital infection the agglutination titre generally, if not always, declines very rapidly and reaches zero before the calf is a year old. In No. 10, however, agglutination was still complete in 1 : 100 and nearly complete in 1 : 200 when the heifer was over 16 months old.

The last point to which attention need be called is the slowness of the recovery in many of the cows. The evidence of this is shown in the following table. Notable examples are found in Nos. 13, 22, 28, 31 and 38. It may be mentioned in this connection that at the time when the herd first came under observation the opinion was still held that the majority of cows recover within a few months after abortion, and the owner was therefore encouraged to hope that the isolation of reacting animals would only be temporary. The experience gained in this and many other herds proved that in a large proportion of cases recovery is very slow. That is the greatest obstacle to the eradication of the disease from valuable pedigree herds.

Table Showing Results of Successive Tests.

No. 1.	20.1.13.	½, 1 and 2 comp.
	7.6.13.	½, 1 and 2 comp.
	11.5.14.	½, 1 and 2 comp.
No. 4.	11.5.14.	½, 1 and 2 comp.
	27.10.14.	½ nearly comp., 1 adv., 2 apprec.
No. 5.	11.5.14.	½, 1 and 2 comp.
	27.10.14.	½ comp., 1 nearly comp., 2 adv.
No. 7.	11.5.14.	½, 1 and 2 comp.
	27.10.14.	½, 1 and 2 comp.
	10.5.16.	½ comp., 1 apprec., 2 neg.
No. 10.	(Heifer one year old and not served.)	
	11.5.14.	½, 1 and 2 comp.
	27.10.14.	½ and 1 comp., 2 nearly comp.
No. 12.	27.10.14.	½ comp., 1 and 2 adv.
	10.5.16.	½ dist., 1 neg.
No. 13.	27.10.14.	½, 1 and 2 comp.
	10.5.16.	½, 1 and 2 comp.
No. 15.	27.4.15.	½, 1 and 2 comp.
	10.5.16.	½, 1 and 2 comp.
	1.1.18.	¼ dist., ½ adv., 1 and 2 nearly comp. (Paradoxal.)
No. 18.	27.4.15.	½, 1 and 2 comp.
	10.5.16.	½ comp., 1 adv., 2 neg.
No. 22.	10.5.16.	½, 1 and 2 comp.
	1.1.18.	½ nearly comp., 1 apprec., 2 neg.
	14.10.18.	¼ nearly comp., ½ adv.
	12.5.19.	¼ comp., ½ nearly comp.
No. 23.	10.5.16.	½, 1 and 2 comp.
	1.1.18.	¼ comp., ½ nearly comp., 1 apprec., 2 neg.
	14.10.18.	¼ nearly comp., ½ adv.
No. 28.	1.1.18.	1 comp., 2 nearly comp.
	14.10.18.	¼ and ½ comp.
	12.5.19.	¼ and ½ comp.
	16.6.20.	¼ and ½ comp.
No. 31.	14.10.18.	¼ and ½ comp.
	12.5.19.	¼ and ½ comp.
	16.6.20.	¼ and ½ comp.
	13.2.21.	¼ and ½ nearly comp.
No. 32.	14.10.18.	¼ and ½ comp.
	12.5.19.	¼ and ½ comp.
	16.6.20.	¼ and ½ comp.
No. 35.	(Calf born 22.3.19. Dam No. 23.)	
	12.5.19.	¼ and ½ nearly comp., graded.
	5.3.21.	¼ and ½ neg.
No. 38.	5.6.21.	¼ adv., ½ apprec.
	13.1.22.	¼ comp., ½ adv.
	14.12.22.	¼ and ½ comp.
	18.7.23.	¼ and ½ comp.
No. 39.	5.6.21.	¼ and ½ both comp.
	13.1.22.	¼ and ½ both comp.
	14.12.22.	½ comp., 1 nearly comp., 2 neg.
No. 40.	13.1.22.	¼ and ½ comp.
	14.12.22.	¼ comp., ½ nearly comp.

Building up an Abortion-free Herd.

At the Research Institute of the Royal Veterinary College striking evidence of the value of the agglutination test was also provided by showing that it could be employed with success to build up an entirely new herd, to be established with all the animals free from the infection of contagious abortion.

The herd to be created was one entirely composed of pedigree animals of a particular breed and the animals desired were purchased on condition that blood might be taken from them for the purpose of a test. Very high prices were offered for some of them (up to several hundred pounds in one case), on that condition, and fortunately for the owners of the new herd some of the animals failed to pass the test.

In this way a herd of about 20 animals was gradually created. Each animal was moved from the farm at which it had been bred to the one at which the new herd was to be maintained, which for many of the animals was a different county. At this farm the premises had been kept vacant for some considerable time and it was intended to keep no other cattle there except those of the new herd. It is hardly necessary to say that after the full complement of animals had been obtained a further test of all the animals was made after an interval to ensure that the precautions to exclude the purchase of an infected animal had not failed. The attempt was completely successful.

STUDIES ON BOVINE MASTITIS.

XII.—MASTITIS DUE TO STAPHYLOCOCCI.

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It is generally held that, compared with streptococci, staphylococci are not of great importance in bovine mastitis. While it is known that staphylococci of the udder are occasionally responsible for a particularly severe and gangrenous form of bovine mastitis, their importance does not rest solely upon this fact. In connection with the control of chronic streptococcus mastitis, S. J. Edwards (1934) has pointed out that " haemolytic " staphylococci (*Staph. aureus*) may be a complicating factor in diagnosis where sole reliance is placed on field or so-called indirect tests. These organisms, by producing a mild inflammation of the udder tissue, may cause the milk to be abnormally alkaline and may lead to an increased output of leucocytes. Cows so affected may, therefore, be eliminated unnecessarily. Again, staphylococci, which there is reason to believe originate from udder milk, occasionally cause outbreaks of boils on the skin of the udder* (Jones, 1918, and others). A parallel case is seen in man where staphylococcus infection about the mouth and nares may originate from staphylococci residing in the nasal cavity. There is further a possibility that *Staph. aureus* through its toxin may predispose the udder tissue to attack by other potential pathogens that may be present. Finally, it has been proved that staphylococci are sometimes responsible for food-poisoning in man and there is evidence that staphylococci from milk are sometimes active in this respect.

Literature.

It has long been recognised by Steck (1920, 1930) that, while certain udder quarters tend to remain relatively germ-free for long periods, others continue to harbour relatively large numbers of bacteria, including staphylococci (*aureus* and *albus*), and that such organisms are capable of setting up a mild inflammatory reaction in the gland. The degree of reaction, as indicated by the leucocyte content (especially neutrophils) of the milk, may vary from time to time, but tends to be directly correlated with the number of bacteria present (especially is this so with the more pathogenic forms such as streptococci, *Staph. aureus* and *albus*) and the amount of irritation set up by their metabolic products (Steck, 1930; Bachmann, 1932). Alice Evans (1916) made a study of the micrococci which occur in normal udder milk. One hundred and ninety-two milk samples from 161 cows were examined and micrococci were isolated from 113 (58 per cent.) of the samples. Most of the strains—the actual number being 185—could be placed in a group

* An instance of this was recently brought to my notice by Mr. J. W. Hall Masheter, M.R.C.V.S. The staphylococci isolated from the boils were identical biochemically and in toxigenic qualities with those commonly occurring in the udder.

possessing characters similar to those of the pyogenic staphylococci, while roughly one-half of the cultures belonging to this group were of the *aureus* variety. Tests for hæmolysis were made on *ox* blood agar plates. (As will be shown later, ox- or sheep-blood agar is suitable for differential purposes, whereas horse-blood agar is not.) Many of the cultures were actively hæmolytic and there was a tendency for this property to be correlated with pigment formation, fermentation of mannite and, so far as tests were made, with virulence for rabbits. Diernhofer (1933) reported the presence of micrococci in at least one-quarter in 135 cows out of 386 examined (35 per cent.). The bacteria belonged to at least 12 different groups, of which one was clearly *Staph. aureus*, and the irritation produced by this organism might resemble that caused by *Str. agalactiæ*. It had previously been found by A. F. Breed (1928), on the basis of biochemical reactions, that the micrococci of the normal udder could be arranged in at least 12 species, of which the commonest was *M. aureus*.

As regards the pathology of staphylococcus mastitis, Minett, Stableforth and Edwards (1929), in the first paper of this series, referred to some of the earlier literature, described 6 acute cases, and mentioned the main bacteriological features of the organism isolated. Parshall (1934) refers to acute gangrenous mastitis in California. Out of 22 cases, mostly in young lactating cows, 15 occurred during the cold rainy season. The cases arose sporadically and were often accompanied by subcutaneous œdema over the abdomen. Bacteriological examination of 12 cases showed that *Staph. aureus* was present in 11, but in 9 of these anærobic infection—mainly with *Cl. welchii*—was also present. Mammary inoculation experiments with the organisms isolated were described, the results tending to be most severe when cultures of both groups of organisms were injected. Klimmer (1930) in Germany recognises the occurrence in cows of acute mastitis due to staphylococci. Albiston (1930) states that in Victoria (Australia) mastitis due to staphylococci is comparatively uncommon. The disease, when it does occur, is either very acute or of the chronic botriomycotic type. Little and Foley (1935) described 9 acute cases of bovine mastitis due to staphylococci. They found that certain staphylococci isolated from the milk of normal udders, from fatal cases of mastitis and from superficial abscesses of the skin of the udder, formed a definite group with common characters which agreed with those of *M. aureus*. The organisms possessed slight if any hæmolytic power, but presumably this was in horse-blood agar. The authors were able to set up acute fatal mastitis in a cow by injecting up the teat canal several times at intervals small amounts of staphylococcus culture from an udder skin abscess. Crabtree and Litterer (1934), in investigating a series of outbreaks of food-poisoning evidently associated with two cases of staphylococcus infection of the udder, called attention to the great variation in the numbers of the organisms excreted in the milk from time to time. The authors supposed the variation to be due to the formation in the gland of pus pockets, from which " showers " of staphylococci were liberated at intervals. The experience of Plastridge *et al.* (1936) is that mastitis associated with " hæmolytic " staphylococci (ox-blood agar may be assumed) is usually transitory and is limited, with few exceptions, to an abnormally high leucocyte count.

The bacteriology of " hæmolytic " staphylococci from various animal sources was studied by Minett (1936). In view of the fact that Glenny and Stevens (1935) had established that staphylococci can produce two different kinds of toxins, designated α and β, for which there are corresponding antibodies, special attention was paid to the toxigenicity of the animal

strains. It was found that the production of β toxin is a characteristic feature of pathogenic staphylococci from animals. No relation could be noted between the strength of toxin and the source of the strain, *e.g.* staphylococci from clinically normal udders may produce as much α or β toxin as strains from obvious cases of mastitis, or even more. The production of β toxin by staphylococci of bovine origin has recently been confirmed by Bryce and Rountree (1936). The last-named authors, and also Naidu (1934) give a description of the appearance of staphylococcus cultures on sheep-blood agar.

The following observations concern the frequency with which stapylohcocci are associated with mastitis in cows.

Jones (1918), among 81 cases of mastitis, found 24 in which micrococci (staphylococci) were the cause. Cultures were non-hæmolytic but it may be assumed that horse-blood agar was used for isolation. Clinically, cases were frequently mild and later staphylococci might disappear from the milk, but sometimes there was acute inflammation which became chronic, with persistence of the organisms, atrophy and induration of the quarter. Carpenter (1925) examined samples of altered udder secretion from 150 cases of mastitis, 9 of which were associated with staphylococci. Hardenbergh and Schlotthauer (1927), among 68 cases of mastitis in 4 herds, found 7 which they considered were due to staphylococci, 5 of these occurring on one farm. Blood agar was used for isolation by Carpenter and by Hardenbergh and Schlotthauer, but the kind of blood used was not specified. Richter and Demmel (1928) mention that 3 cases of mastitis out of 61 examined were due to staphylococci. Rolle (1932) made a systematic examination of 200 cases of mastitis and found 11 per cent. to be due to micrococci. Plastridge *et al.* (1934), in periodic examinations of approximately 200 cows, found that about 10 per cent. of abnormal udder conditions were apparently due to staphylococci. The authors used ox-blood agar for isolation. Hopkirk (1935) states that in some herds in New Zealand staphylococcus mastitis is as common as the streptococcus form and is indistinguishable from it except by cultural tests. About 30 per cent. of cases of mastitis are considered to be due to staphylococci, the latter being hæmolytic in ox-blood agar. Micrococci—that is, non-hæmolytic staphylococci—also are occasionally mildly pathogenic and are associated with a definitely increased output of leucocytes. Gwatkin, Hadwen, and LeGard (1936) examined 143 cases of mastitis, and considered that 30 of them were due to staphylococci. Twenty of the 30 cases occurred in two herds ; hence the authors make the important observation that staphylococcus mastitis may arise not only sporadically but also as a herd infection, frequently taking the form of a chronic induration of one or more quarters without constitutional disturbance of the animal. The organisms were isolated on agar containing rabbit-blood, but, since only 31 of 46 staphylococcus strains recovered from the cases of mastitis produced toxin by Burnet's soft agar-CO_2 method, it seems somewhat doubtful whether the remaining strains were responsible for the mastitis.

Cases of Staphylococcus Mastitis Investigated.

Acute cases.

Since the first paper in this series of studies, 10 acute cases due due to staphylococci* have been investigated, 9 of which showed

* Three other acute cases were due to *Bact. coli*, *Bact. aerogenes*, and *Cl. welchii* respectively.

definite gangrenous changes in one or sometimes two quarters, and 5 of which died. Four of the 9 cases occurred at about the time of calving, while the other 5 appeared 4 to 6 months after calving, three of the latter being fatal. Most of the cases were in older cows, but two fatal cases occurred in first calvers, and one of these two animals, it is important to note, had calved only 24 hours before the onset of symptoms. In most of the cases systemic disturbance was severe and sometimes there was considerable subcutaneous oedema over the abdominal wall. The secretion from the gangrenous cases was a reddish-brown watery fluid, containing coagulated masses and shreds of necrotic tissue. Staphylococci were seen in smears of the centrifuged deposit from this fluid, and by plating in sheep- or ox-blood agar the numbers of staphylococci present in the secretion were found to be relatively enormous. In 7 cases a search was made for anaerobic bacteria ; in 3 of these by putting up glucose agar shake cultures and in 4 by sowing into cooked meat medium under vaseline and also inoculating guinea-pigs subcutaneously. In 5 of these 7 cases there was no evidence at all that bacteria other than staphylococci were responsible for the mastitis. In one of the two remaining cases a few Gram-positive bacilli were seen in the secretion together with very large numbers of staphylococci. Microscopical examination of the affected quarter in this case showed masses of staphylococci situated in necrotic material derived from the secreting ducts or in the cell areas which had replaced them. In the other case the only organisms seen in the secretion were staphylococci in enormous numbers, but gas-producing bacilli appeared in relatively small numbers in the meat medium. Since, however, the original secretion produced only a local abscess in the guinea-pig, it was assumed that the gas-producing organisms were unimportant.

One case requires special mention. The cow calved on 27.1.37, two days later the hind quarters were noticed to be swollen and from them with difficulty a small quantity of brownish fluid was extracted. Judging by microscopical examination and cultivation in ox-blood agar, staphylococci in enormous numbers were *the only organisms present*. On 9.2.37 the cow was given 10 c.c. concentrated staphylococcus antitoxin (Burroughs, Wellcome & Co.) and on 22.2.37 she was sent to the Institute. All four quarters of the udder were then greatly swollen and secretion from each of them was examined microscopically and plated in ox-blood agar. All four samples were now found to contain enormous numbers of *C. pyogenes*, but considerable numbers of staphylococci were still present in the two hind quarters. In this case, therefore, a violent *C. pyogenes* infection was superimposed on the staphylococcus mastitis, and this gives some reason for the suggestion that in the pathogenesis of some udder infections an association between different bacterial species may be important. The cow was slaughtered on 25.2.37, and *post-mortem* the only abnormality seen, apart from that in the udder, was cloudy swelling of the liver.

Chronic cases.

Five cows affected with the disease in a chronic form were kept at the Institute for observation, but a detailed examination was made

on only three of them (numbers 3, 4 and 5). Unfortunately, cows 3 and 5 proved less suitable than had been expected. With cow 6, the udder alone was received for study.

The clinical condition of the affected quarter was noted from time to time immediately after milking. The milk from the individual quarters of the udder was taken into a four-chambered pail, in order that the yield of each quarter might be known. Using samples of fore milk taken at the morning milking, estimations were made by current methods for specific gravity, fat, solids-not-fat and total solids.* Note was taken of the gross appearance of the milk, of its keeping quality at 22° C. (as judged at intervals by appearance, taste, and coagulability on boiling), of its reaction to brom-cresol-purple test papers, of the amount of sediment on centrifuging 10 c.c. volumes. Estimations of the leucocyte content were made by a modified Breed method—a loopful of gravity cream being spread on a slide over an area of approximately 1 sq. cm., the film being defatted with xylol and stained with thionin.

Within three hours of collecting the samples, the total viable count was taken by plating the fore milk in lemco agar to which 0·5 per cent. milk had been added, the colonies being counted after 48 hours at 37° C. The method of examining for *Staph. aureus*, carried out as a rule every 3 or 4 days with fore milk, mid milk, and strippings, was as follows. 80 c.c. of the fore milk was stored in the cold for 3 hours, and 0·5 c.c. of the gravity cream then removed to 9 c.c. saline ; 10 c.c. of the remaining milk was centrifuged and the deposit mixed with a similar quantity of saline. 1·0 c.c. amounts of whole milk (fore, mid, and strip) were mixed with 9 c.c. vols. of saline. From the tubes so prepared, one further dilution was made by transferring 1·0 c.c. to 9·0 c.c. saline, 1·0 c.c. of each dilution being then mixed with the medium in a Petri dish. One graduated pipette was used for each portion of the milk examined. The medium consisted of agar containing 5 per cent. defibrinated blood, usually from a young heifer, occasionally from a sheep, and plates were examined after 24 hours at 37° C.

When there was an opportunity of examining the udder after death, each quarter was cut into thin slices and a number of representative pieces taken for histological examination.

The following particulars of these cases are given. Additional information will be found below under the appropriate heading.

Cow 1 was infected in the L.F. quarter. During 6 weeks' observation 1·0 c.c. of the fore milk was plated on 30 occasions. On 15 of these—usually during the second half of the experiment—no staphylococcus colonies were seen ; at other times the number of colonies on the first plate (0·1 c.c. milk) varied from a few to innumerable. The milk from the L.F. quarter showed no other abnormality and there was no clinical disturbance in the quarter. Histological examination, however, of the L.F. quarter showed an infiltration of fibroblasts between the acini.

Cow 2 was affected with chronic streptococcus mastitis in the R.F. quarter and was excreting *Staph. aureus* from the L.F. quarter. Whole milk, gravity cream, and centrifuge sediment from the L.F. quarter were plated in sheep-blood agar on 21 occasions during two months. The

* These analyses were undertaken in the Central Laboratories of United Dairies Limited.

organisms were always found in these samples, except on three occasions in the whole milk and once in the deposit.

Cow 3 gave birth to her first calf on 15.10.35. Between 7.7.36 and 26.10.36, when the cow went dry, platings were made on 31 occasions from the infected R.H. quarter, and *Staph. aureus* was detected on 23 of these occasions. During the month before the cow was finally dried off, the organisms were usually not detected or detected only in some portions of the milk. The cow calved again on 8.2.37, and it was then ascertained as a result of 17 platings (between 11.2.37 and 16.4.37) that the R.H. quarter was excreting staphylococci only exceptionally. A slight infection was also found in the other three quarters, the largest number of staphylococci being present in the R.F. milk. On 15 occasions during the second lactation films were examined for leucocytes, but no obvious increase was seen in any quarter.

During the first lactation, the R.H. quarter was slightly smaller and was firmer than the L.H. After the second calving, no difference in the size of the hind quarters could be appreciated but the R.H. remained slightly indurated.

The cow was slaughtered on 20.4.37. On section the udder showed no change visible to the naked eye. Microscopically, the R.F. quarter was the only one showing abnormality, there being collections of neutrophils in some of the acini.

Cow 4 gave birth to her fifth calf on 2.9.36. Between 1.10.36 and 16.4.37 platings were made on 49 occasions from the infected R.H. quarter, and at rather wider intervals from the other quarters. *Staph. aureus* was detected in the R.H. milk on every occasion except one, but never in milk from the other three quarters. On 11 of the 49 occasions the staphylococci failed to develop on plates sown from certain portions of the same sample, whole milk (fore and mid) being especially liable to fail in this respect. On 36 occasions between 27.11.36 and 16.4.37 films of cream were examined for leucocytes ; almost invariably conditions in the L.F. and L.H. quarters were normal, whereas in the R.F. and R.H. quarters there was nearly always an excess and sometimes a great excess of neutrophils. Apparently, the cause of this cell increase in the R.F. milk was a streptococcus of the enterococcus-lactis group.

In the R.H. quarter there was a diffuse induration, easily palpable as soon as the cow had been milked, but there was no appreciable difference in size between the two hind quarters. The R.F. quarter was slightly larger and firmer than the L.F. and on two occasions the R.F. milk was noted to be yellowish and contained clots.

Cow 4 was slaughtered on 20.4.37. The only quarter showing gross evidence of disease was the R.H. On section, this quarter was obviously indurated and much of its tissue was brownish in colour. Microscopically, in certain areas the acini were filled with solid masses of neutrophils and these cells were also infiltrating the interacinar septa. These were greatly thickened, mainly as a result of increased fibroblast activity. In other parts the disease process was more advanced, there being a large increase of fully-formed connective tissue and atrophy of the secreting tissues (Fig. 1). Fig. 2 shows the appearance of the L.H. quarter with its normal structure.

Cow 5 gave birth to her third calf on 25.6.36. Between 24.11.36 and 16.4.37 platings were made on 36 occasions from the R.H. quarter—the one mainly involved—and *Staph. aureus* was detected on every occasion and in every portion of the sample. The three other quarters, especially the L.H., were also infected with these organisms. Infection of the L.H.,

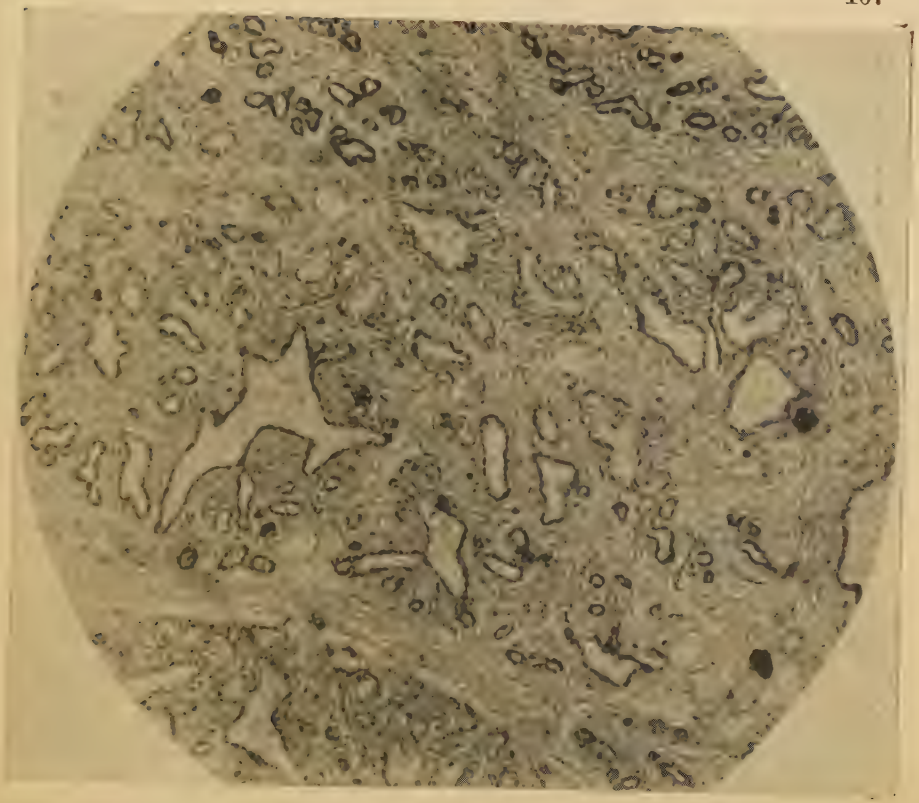

FIG. 1. Cow 4 R.H. Connective tissue increase from staph. infection. × 100.

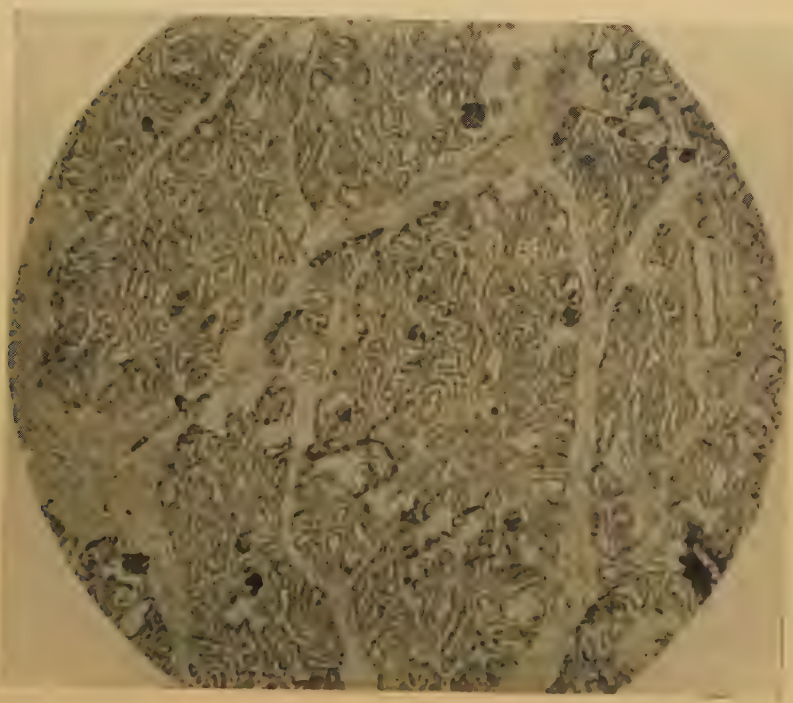

FIG. 2. Cow 4 L.H. Normal tissue. × 100.

however, was much less than that in the R.H., and some portions of the samples commonly failed to show staphylococci. Films of cream were examined on 25 occasions between 28.11.36 and 16.4.37. No increase in cells was noted except in the R.H., where there was nearly always a great excess of neutrophils.

In the R.H. quarter there was a very definite diffuse induration, but no difference in size was apparent between the two hind quarters.

Cow 6. The udder was obtained for examination because the animal had shown an unusually high blood antitoxin titre (about 36 α units per c.c.) and because the owner stated that there had been trouble in the L.H. quarter and great decrease in yield from it during the first and second lactations. At the time of slaughter the L.H. quarter was firm, reduced in size and nearly dry, while the other three quarters were still giving milk. The small amount of milk in the L.H. cistern was yellowish and creamy and yielded *Staph. aureus* in pure culture. The cow had previously passed two cultural tests for streptococci. Histological examination of the quarter showed the production of much new connective tissue and in some places between the acini there was considerable infiltration of fibroblasts.

The Milk Secretion in Chronic Cases.

Milk Yield.

Koestler *et al.* (1926) state that it is usually accepted that the quarters on the right side give rather more milk than those on the left, and that the fore quarters give less milk than the hind ones. A test of 50 healthy cows, made by these authors, showed that the mean yield of individual quarters, expressed as a percentage of the total yield, was as follows:

L.F. 19·8	L.H. 29·1
R.F. 21·8	R.H. 29·3

In Table I are shown data relating to the yield of the individual quarters of cows 3, 4 and 5. The differences in yield of corresponding quarters are set out in Table II.

TABLE I.

Cow and Quarter.	*Mean Daily Yield (c.c.)*	*Standard Deviation.*	*Range of Yield (c.c.).*	*Percentage of Total (observed) Yield.*
3 (*a*)				
L.F.	1,170 ± 21	110	880–1,310	23·6
R.F.	1,659 ± 28	155	1,320–1,930	33·3
L.H.	1,542 ± 32	175	1,225–1,820	31·9
R.H.	573 ± 16	89	355– 745	11·2
3 (*b*)				
L.F.	2,858 ± 50	251	2,240–3,240	22·3
R.F.	2,420 ± 31	153	1,950–2,600	18·9
L.H.	3,742 ± 44	219	3,290–4,240	29·4
R.H.	3,760 ± 43	217	3,510–4,000	29·4
4				
L.F.	3,270 ± 78	437	1,930–4,270	22·8
R.F.	3,224 ± 61	493	2,280–4,240	22·4
L.H.	4,261 ± 81	654	3,010–5,875	29·6
R.H.	3,634 ± 77	619	2,160–5,010	25·2
5				
L.F.	567 ± 17	106	335– 930	12·6
R.F.	620 ± 19	117	340– 840	13·8
L.H.	1,705 ± 43	272	1,280–2,440	38·0
R.H.	1,591 ± 52	329	1,010–2,420	35·6

3 (*a*) = before second calf. 3 (*b*) = after second calf.

The number of times and the period over which the yield was measured were as follows :—

Cow 3	(*a*)	20	times in	74 days.
	(*b*)	25	„	46 „
„ 4		65	„	116 „
„ 5		40	„	79 „

TABLE II.

Cow.	*Quarters.*	*Differences of Mean Daily Yield (a).*	*Standard Error of Difference (b).*	*Ratio : $\frac{(a)}{(b)}$*
3 (*a*)	R.F.–L.F.	489	35	14
	L.H.–R.H.	969	36	27
3 (*b*)	L.F.–R.F.	438	59	7·4
	R.H.–L.H.	18	62	0·3
4	L.F.–R.F.	46	98	0·5
	L.H.–R.H.	627	112	5·6
5	R.F.–L.F.	53	25	2·1
	L.H.–R.H.	114	67	1·7

The data shown are, of course, little more than suggestive. But it will be seen that with cows 3 (before second calf) and 4, the reduction in yield from the affected R.H. quarters is significant, but this is not the case with the R.H. of cow 5, probably because the L.H. of this animal was also affected. With cow 3, however, there are significant differences in yield from the fore quarters—the R.F. showing the greater yield before calving and the L.F. after. The reasons for this are not clear. Before calving, the fore quarters of this cow were only examined on two occasions and with negative results ; after calving, both fore quarters were known to be carrying staphylococci, the R.F. being the most heavily infected and found later to be showing distinct histological abnormalities.

Physical and Chemical Examination.

Examinations of a large series of routine laboratory records showed that the milk from staphylococcus-infected quarters usually was not grossly changed in appearance except that occasionally it was " watery." Quite frequently, however, the reaction was too alkaline and there was a slightly increased sediment on centrifuging. These changes tended to be most common in milks showing the greatest number of staphylococci.

The general truth of these remarks was borne out by the more detailed examinations with cows 3, 4 and 5. The milk from the affected quarters of these animals was almost always unchanged to the naked eye. On one occasion the R.H. milk from cow 4, although normal in appearance, gave after a few hours' standing a heavy, slimy deposit containing masses of leucocytes with a few cocci.

The keeping quality of the milk, as judged by the usual standards, was not altered to any important extent by the presence of *Staph. aureus*, but on a number of occasions with cows 4 and 5, milk from the R.F. and R.H. quarters was described as " salty " on arrival at the laboratory.

In the estimations for total solids, fat, solids-not-fat, and specific gravity, the figures for specific gravity and solids-not-fat were the only ones showing significant differences. These are reproduced in Tables III and IV.

TABLE III.

Cow and Quarter.		Specific Gravity.			Solids-not-fat.		
		Mean.	Standard Deviation.	Range.	Mean.	Standard Deviation.	Range.
3 (after calving)	L.F.	1,032·6 ± 0·2	1·1	1,030·3–1,035·1	8·49 ± 0·04	0·22	8·14–8·97
	R.F.	1,032·3 ± 0·2	1·3	1,030·5–1,035·3	8·52 ± 0·04	0·24	8·15–9·06
	L.H.	1,032·9 ± 0·2	1·4	1,029·3–1,035·4	8·48 ± 0·04	0·22	8·10–9·02
	R.H.	1,032·9 ± 0·2	0·9	1,030·6–1,035·0	8·52 ± 0·04	0·20	8·10–8·99
4	R.F.	1,033·4 ± 0·2	1·1	1,029·6–1,036·7	8·85 ± 0·03	0·20	8·52–9·65
	L.F.	1,033·4 ± 0·1	0·9	1,031·4–1,035·8	8·84 ± 0·03	0·19	8·39–9·31
	L.H.	1,033·6 ± 0·1	1·0	1,031·5–1,035·8	8·83 ± 0·03	0·19	8·25–9·46
	R.H.	1,032·3 ± 0·1	1·0	1,029·2–1,034·4	8·57 ± 0·03	0·21	8·03–9·07
5	L.F.	1,032·5 ± 0·2	1·1	1,030·5–1,036·0	8·95 ± 0·03	0·19	8·55–9·48
	R.F.	1,031·7 ± 0·1	0·9	1,029·7–1,034·4	8·75 ± 0·03	0·22	8·20–9·78
	L.H.	1,033·8 ± 0·2	1·4	1,027·8–1,037·0	9·11 ± 0·03	0·21	8·55–9·77
	R.H.	1,032·9 ± 0·2	1·5	1,028·7–1,036·4	8·91 ± 0·05	0·32	8·12–9·73

TABLE IV.

Cow and Quarter.		Specific Gravity.			Solids-not-fat.		
		Diff. in Means (a).	S.E. of Diff. (b).	Ratio : $\frac{(a)}{(b)}$	Diff. in Means (a).	S.E. of Diff. (b).	Ratio : $\frac{(a)}{(b)}$
3	L.F.–R.F.	0·3	0·3	1·0	(R.F.–L.F.) 0·03	0·06	0·5
	L.H.–R.H.	0·0	0·3	0·0	(R.H.–L.H. 0·04	0·06	0·7
4	R.F.–L.F.	0·0	0·2	0·0	0·01	0·04	0·3
	L.H.–R.H.	1·3	0·1	13	0·26	0·04	7
5	L.F.–R.F.	0·8	0·2	4	0·2	0·04	5
	L.H.–R.H.	0·9	0·3	3	0·2	0·06	3

Number of observations with cows 3, 4 and 5 were 27, 54 and 51 respectively.

The significant differences observed are, therefore, as follows. The R.H. quarter of cow 4 is significantly lower than the L.H. both in specific gravity and solids-not-fat. In both these respects also the R.F. and R.H. quarters of cow 5 are significantly lower than the L.F. and L.H. quarters, but the real interpretation of this finding is uncertain because all four quarters were infected with *Staph. aureus.*

In connection with these analyses, the work of the following authors is relevant. Mattick and Hallett (1929) studied the milk from individual quarters of a cow during her lactation period of 11 months. It was noted among other things that the differences in the solids-not-fat content of the four quarters were not significant.

Peskett and Folley (1933) expressed the opinion that deficiency of non-fatty solids in milk may be due in some cases to dilution of a normal secretion in the udder by a transudate-like fluid, such as may result from a mild chronic form of mastitis. The secretion of milk containing an abnormally low proportion of solids-not-fat may therefore represent a pathological rather than a physiological condition. The findings with cow 4, at any rate, go to support that opinion in respect of the mild mastitis due to *Staph. aureus.*

Bacteriological Examination.

The total viable count with the milk of cows 4 and 5 varied considerably from time to time and from one quarter to another, so that no relationship could be seen between the total count and the numbers of *Staph. aureus* being excreted.

In the special examination for *Staph. aureus* one of the outstanding features was the enormous variation in the count from time to time even at short intervals. For instance, with cow 4 on one occasion staphylococci grew from the end milk only and there were only two colonies on the first plate. Three days later, the counts on the first plates from each portion were less than 10, while two days later, still the counts on the corresponding plates were innumerable. The operations were always carried out by the same skilled technician and there was no reason to suppose that such results were due to error.

From the figures available information was obtained as to the most suitable portion of the milk to examine from the point of view of diagnosis. Considering the results with the first dilution plate in each series, but ignoring the number of *Staph. aureus* colonies present, all portions of the milk were equally satisfactory, but when the viable count of these organisms was taken into consideration, cream was by far the most satisfactory (Table V).

Thus, the cream gave the highest count in 71 per cent. of samples, and end milk in 26 per cent. The count was higher more frequently with the end milk than with the fore milk, a finding contrary to that of Little and Foley (1935).

TABLE V.

VIABLE COUNT OF *Staph. aureus* FROM DIFFERENT PORTIONS OF THE SAME MILK SAMPLE.

Cow and Quarter.	*Samples Examined.*	*Viable Count* (Staph. aureus) *on first plate with :*				
		Cream.	*Deposit.*	*Fore.*	*Mid.*	*End.*
3 R.H. (first lactation)	17	13	2	0	1	2
4 R.H.	46	27	7	4	5	17
5 R.H.	36	31	2	9	0	7
	99	71	11	13	6	26

Incidence of Udder Infection with Staph. aureus.

Ten c.c. samples of milk from the separate quarters of 415 cows belonging to five herds were centrifuged and the deposit plated in ox- or sheep-blood agar, with the result that *Staph. aureus* was found in the milk of 145 cows (34·9 per cent.). Infection appeared to be more common in the hind quarters than in the fore ones. The distribution according to herds was as follows :—

Herd.	*Number of Cows.*	*Number Infected.*
I	59	29
A	186	62
R	116	31
X	23	3
Y	31	20
	415	145

None of these animals was affected with streptococcus mastitis. On the first plate of the series, *i.e.*, on one sown with half the deposit from 10 c.c. milk, there were often only a few colonies, at other times the colonies were innumerable with smaller amounts of deposit. In a large proportion of cases the nature of the staphylococci present was controlled by testing the toxigenicity of the cultures isolated.

It will be noted that the average incidence figure (34·9 per cent.) scarcely differs from that given by Diernhofer (1933), but two points should be mentioned. Firstly, the incidence figure is certainly a minimal one, because in most cases the milk was taken only once for cultural examination. Secondly, the incidence figure varies considerably from one herd to another ; in herd Y, for instance, 20 cows out of 31 were infected.

It was not possible to estimate from the figures the incidence of infection according to age, because the older animals may have been infected in earlier lactations. Fifty-six of the animals in herd A, however, were first-calvers and the incidence of infection among these was 23 per cent. This would indicate that infection is frequent during the first lactation.

As a means of gaining further information about the importance of staphylococcus mastitis in practice, herd Y was examined in

company with Mr. J. R. Rider, M.R.C.V.S. In 19 out of 26 quarters infected with *Staph. aureus* there was palpable induration and in 2 of the 19 cases the quarter was distinctly " atrophied." Fifteen of the 19 quarters and 3 of the remaining 7 quarters were excreting staphylococci in very large numbers a week before the clinical examination was made. In this herd also, 8 quarters of 4 cows were infected with β-hæmolytic streptococci; all these quarters were indurated but, so far as could be judged, the induration was not greater than that in the staphylococcus-infected quarters.

Staphylococci of the Cow's Udder.

These can be conveniently placed under two headings, *viz.*, pathogenic staphylococci (*Staph. aureus*) and non-pathogenic staphylococci.

Pathogenic staphylococci.

Growth in blood agar.—As stated previously, for routine purposes, agar poured to a thickness of about 2 mm., and containing about 5 per cent. defibrinated ox- or sheep-blood has been used. The inoculum was placed in the dish and mixed with the medium before it set.

The appearance of the growth depends mainly on two factors, the kind of toxin the culture produces and the kind of blood added to the medium. In my experience the proportion of udder strains which form α toxin only is comparatively small, the great majority producing both α and β, while occasional strains appear to form β toxin only. With horse-blood agar there is no hæmolysis or other change, because horse cells are relatively insusceptible to both toxins. With strains forming α toxin only, there is in rabbit-, sheep- or ox-blood agar after 24 hours at 37° C. more or less complete lysis in the neighbourhood of well-separated deep colonies and complete lysis beneath and around surface ones. With strains forming solely or mainly β toxin, well-separated colonies in sheep- or ox-blood are surrounded by a sharply-defined zone, up to 15 mm. in diameter, in which the blood pigment is darkened in colour. Where both α and β toxins are produced, the clear, central zone is surrounded by a much broader belt of darkened blood—an appearance which is very valuable for diagnosis (Fig. 3). With rabbit-blood agar the effect of the β toxin does not appear, because rabbit cells are insusceptible to this toxin. On the other hand, since rabbit cells are more susceptible than sheep cells to the α toxin, the zone of lysis next to the colony in this case tends to be rather broader in rabbit-blood agar. If after incubation the plates are allowed to stand at room temperature or in the cold for 24 hours, the α zones tend to become rather wider and the β zones become clearer (especially in the cold), while at room temperature a second narrower darkened zone appears outside the first (Fig. 4). Growth

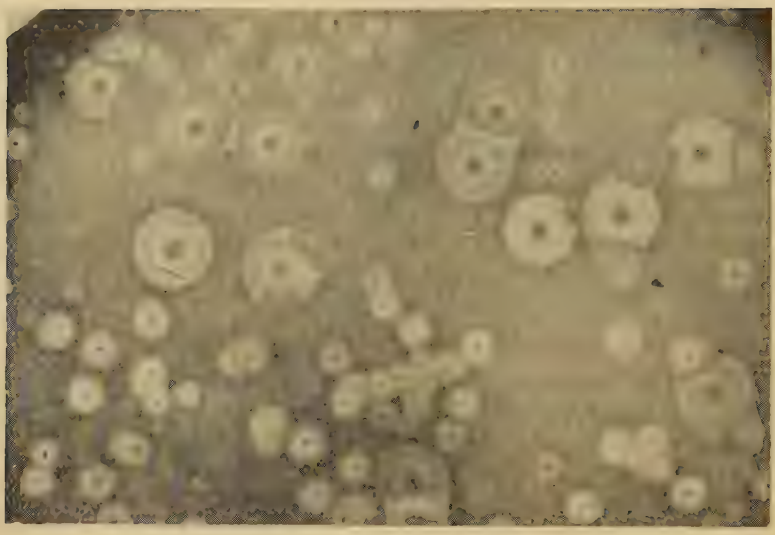

FIG. 3. Cow 5 R.H. milk. Ox-blood agar, 37°C. 24 hours. Staph. colonies, deep and surface.

FIG. 4. *Staph. aureus* from udder. Ox-blood agar, 37°C. 24 hours. 24 hours on bench. Surface colonies with outer darkened zone.

is inhibited in blood agar to which crystal violet has been added to a concentration of 1 in 500,000.

Effect of natural antitoxin.—The appearances described above are obtained when the blood used contains no appreciable natural antitoxin. An experiment was carried out to ascertain the effect of using blood containing antitoxin in amounts such as may be present in cow blood. Three samples of citrated blood were used: sample A was from a first-calf heifer and the plasma contained 1·6 units α and $<0{\cdot}34\gamma$ β units antitoxin per c.c., sample B was from a cow infected with staphylococcus mastitis and the plasma contained 9·0 α units and $3{\cdot}6\gamma$ β units antitoxin per c.c., while sample C was made by adding 1 part of a standard serum (580 α units and 40γ β units per c.c.) to 28 parts of sample A. The citrated plasma from sample C was subsequently estimated to contain 18·0 α units and $1{\cdot}8\gamma$ β units antitoxin per c.c. Seven staphylococcus strains were used for the test, *viz.*, 4α, $2\alpha+\beta$, and 1β strain, and the result clearly indicated considerable inhibition of hæmolysis by sample C and definite inhibition by sample B. The actual results were influenced as would be expected, by the toxigenic capacity of the particular strain, while the effect of the antitoxin was most noticeable about deep colonies. Thus, with some strains, deep colonies in the agar containing sample C were quite non-hæmolytic, whereas with sample A the deep colonies were surrounded by zones of clear lysis. Samples C and B also inhibited more or less completely the β effect.

Non-pathogenic staphylococci.

It did not fall within the scope of this work to make anything approaching an intensive examination of the non-pathogenic staphylococci, or, as some would term them, the micrococci of udder milk. Indeed, to be of real value such an examination should include a very great number of strains and should comprise a study of antigenic structure. The main interest has been to see whether presumably non-pathogenic varieties that can be commonly isolated from normal milk produce lysin in quantity when cultivated on soft agar under CO_2, or whether they give any other indications of pathogenicity. When normal udder milk is plated *in* ox-blood agar, it is common to find the following varieties of micrococcus colonies, (*a*) large, smooth, *white** surface colonies with entire edges, standing on a bed of clear haemolysis, (*b*) surface colonies with a bright *yellow** or fawn tint, resting on hæmolysed zones, (*c*) deep colonies which are non-haemolytic and with at times faint greenish discolouration of the blood (naked eye and low-power examination after 24 hours at 37° C., followed by standing up to 48 hours at room temperature). These on further examination may be found to belong to either (*a*) or (*b*). (*d*) delicate flattened surface colonies or minute deep colonies, surrounded by broad and intensely *green* zones up to 3 mm. in diameter.

* The terms " yellow " and " white," as applied to saprophytic cocci of the udder, are not synonymous with the terms " aureus " and " albus," which are ordinarily reserved for pathogenic forms.

Colonies representative of these types were picked and examined as follows. Colour of growth was noted on agar and ox-blood agar. The strains were tested for lysin production by growth on soft agar under CO_2, the juice expressed from the agar being centrifuged and tested on rabbit and sheep corpuscles. Since under these conditions none of the strains produced lysin, the filtrates were not tested on animals for toxicity. Coagulase activity in rabbit plasma and sometimes in human plasma was tested by the method described by Walston (1935). Cultural characters in gelatin at room temperature and in litmus milk at 37° C. were noted ; also fermentation of salicin and mannite, utilisation of ammonium phosphate, reduction of nitrates, power of growth on ox-blood agar containing crystal violet to a concentration of 1 in 500,000, lipolytic activity in agar containing about 0·2 per cent. sterile cream—this is indicated by a whitening of the medium about the growth. The results are shown in Table VI. For comparison the characters of *Staph. aureus* are included.

The following comments are necessary. One of the deep non-hæmolytic colonies proved on further examination to be a pathogenic strain which produced α toxin only. Sixteen yellow cultures, 13 white cultures and 12 green-producing cultures are represented in Table VI. In addition, there were four yellow or white strains which could not be fitted into the two groups. Colour distinctions between yellow and " aureus " strains were sometimes difficult to make. Although many of the yellow and white strains originated from deep non-haemolytic colonies in ox-blood agar, many of them when transferred to the surface of the same medium produced quite distinct areas of haemolysis. No evidence, however, was obtained that this was due to a specific lysin. The yellow strains had a greater tendency to ferment mannite than the white strains, but action on mannite was often delayed. The failure to liquefy gelatin appeared to be a better criterion of lack of toxigenicity than non-fermentation of mannite. Four of the 15 yellow strains showed some lipolytic activity. No certain agreement could be observed between the yellow or the white cultures and the types of micrococci described by Hucker (1924, 1924a, 1928) though the yellow strains would perhaps be classed as *M. varians*. Two white and yellow strains selected at random did not kill or produce obvious lesions in rabbits when 1·0 c.c. broth culture was injected intravenously.

Broth cultures of the green-producing micrococcus contain relatively large cocci arranged in pairs or as tetrads. The green zones are produced in ox-, sheep-, horse- or rabbit-blood agar, and these zones undergo partial lysis if the plates are left at room temperature for 48 hours. On low power examination deep colonies may then show an *alpha* appearance, but the outer parts of the zones are not free from corpuscles. In blood agar containing crystal violet to a concentration of 1 in 500,000 and æsculin to 1 in 1,000, colonies are black and no hæmolysis of the green zone takes place when the

TABLE VI.

Cultures.	*Lysin.*	*Coagulase.*		*Gelatin.*	*Litmus Milk.*	*Salicin.*	*Mannite.*	*Am. Phosphate.*	*Nitrates.*	*Lipase (cream).*	*Agar Growth.*	*Crystal Violet Blood Agar.*
		Rabbit Plasma.	*Human Plasma.*									
Yellow micrococci	—	—	—	— or tr. liq.	—	—	— (usually)	—	+	— (usually)	abund.	—
White micrococci	—	—	—	— or tr. liq.	AC reduction	—	—	+ (usually)	+	+	do.	—
" Green " micrococci	—	—	—	—	Tr. A	+	+ (usually)	—	—	—	V. slight	+
Aureus ...	+	+	+ variable	+	* AC reduction	—	+	—	+	variable	abund.	—

* A few *aureus* strains from the udder grown in " clean " raw milk at 37° C. produced coagulation and, after 2–4 days, digestion of the clot; if, however, the milk was heated at 100° C. for 30 minutes before being sown, coagulation was not followed by digestion.

cultures are left at room temperature. Growth on agar is slight and not stimulated by the addition of serum.

Except in the case of the *aureus* cultures, none of the soft agar centrifugates prepared from the strains represented in Table VI hæmolysed rabbit or sheep corpuscles at a dilution greater than 1 in 10. Some of them, however, gave hæmolysis at dilutions below 1 in 10, and it was important to determine if possible whether this was due to minimal amounts of toxin. Intradermal inoculation in rabbits of undiluted centrifugates giving this degree of hæmolysis was found to produce some oedema, but there was no necrosis such as may be observed with *aureus* filtrates to a dilution of, say, 1 in 80.

An experiment was set up to determine whether such centrifugates could act as a secondary stimulus in rabbits with a low grade of specific immunity. After samples of blood had been taken, a series of rabbits were injected subcutaneously with a small dose of staphylococcus toxoid*, which was intended to act as a weak primary stimulus. Twenty-one days later they were reinjected subcutaneously in groups of four with soft agar centrifugates prepared from the following strains : a yellow micrococcus, a white micrococcus, a green-producing micrococcus, a strain of *Bact. coli*. A fifth group was reinjected with a Berkefeld filtrate from an *aureus* strain, freshly isolated from milk. A sixth group of two rabbits was reinjected with centrifugate from the unsown agar. All the animals had less than 0·1 unit α antitoxin per c.c. in the blood before injection, but unfortunately only about one-third of them had as much as 0·1 unit after the primary stimulus. After the second injection there was a considerable rise of antitoxin with the *aureus* rabbits (to 2·0, 2·2, 4·5 and 7·0 units respectively), but with most of the others there was no appreciable response. A slight rise in antitoxin (to 0·8, 0·4, and 0·4 units per c.c.) was apparent, however, in one rabbit belonging to each of the three groups treated with the yellow micrococcus, the white micrococcus and the green-producing micrococcus, while a rise to 1·6 units per c.c. had occurred with one of the two rabbits injected with unsown centrifugate. On the whole, the experiment gave no indication that micrococci other than *aureus* can provide a specific secondary stimulus.

Discussion.

It is clear that staphylococci which are hæmolytic in sheep- or ox-blood agar are widespread organisms in the udders of cows, and the pathological changes they may produce vary considerably. The alterations produced are usually chronic in nature and, though less severe than those of chronic streptococcus mastitis, there may be some reduction in the yield and qualitative changes in the milk. In the literature this form has not received wide recognition but as mentioned in the introduction, its importance is in part due to the

* Kindly provided by Miss M. Llewellyn Smith, National Institute for Medical Research.

fact that, without a bacteriological examination, changes in the milk may be wrongly ascribed to streptococci. The ultimate reaction of the tissue is mainly one of the stroma and there is no suppuration in the ordinary sense. Another type of chronic lesion, which appears to be uncommon, at any rate in Europe, is that which has been described as " actinomycotic " or " botriomycotic "—terms which may well be avoided. Finally, there is the well-known though relatively uncommon acute gangrenous lesion, which may be due not only to staphylococci but to other organisms such as Clostridia.

As Evans (1916) found, many of the staphylococci from supposedly normal udders are pathogenic for rabbits. In earlier work on the bacterial flora of the udder, in most of which, of course, simple culture media were used, no clear distinction was drawn between staphylococci which are toxigenic and those which have no such action. The value of blood agar for making this distinction is now established. But whereas, in work with streptococci, horse-blood agar can be used, it is important to emphasise that with staphylococci horse-blood agar is unsuitable, for the reason that horse corpuscles are relatively insusceptible to staphylococcus lysin (toxin), as found long ago by Neisser and Wechsberg (1901). Incidentally, in seeking to determine whether a staphylococcus produces a specific lysin in blood agar, care must be taken that the medium contains no added fat, as, of course, happens when milk is plated. The reason is that, as Orcutt and Howe (1922) showed, some non-hæmolytic staphylococci excrete a lipase, so that if fat is present in the medium, the liberated fatty acid will cause hæmolysis. That staphylococci of the udder which produce no hæmolysis in ox-, sheep- or rabbit-blood agar, and which possess no coagulase activity, are capable of exerting a pathogenic action on this organ would appear to be improbable from analogy and in the light of experience gained in this work. On the other hand, there are found in udder milk staphylococci which form white or yellowish surface colonies on blood agar prepared with ox-, sheep- or rabbit-blood, the colonies resting on a bed of clear hæmolysis after 48 hours at 37° C. There is no evidence that this is due to a specific lysin or that these staphylococci act as irritants to the mammary tissue. It is important, therefore, to notice that the production of hæmolysis, even on rabbit-blood agar, after incubation at 37° C. is not necessarily an indication of α toxin production (cf. Geisse, 1914). Staphylococci which form α and β toxin produce on ox-blood agar (which is free from natural antitoxin) after 24 hours at 37° C. pale fawn colonies resting on a clear zone which is surrounded by an area of altered blood pigment. In this area hæmolysis, due to the β component, becomes evident on refrigeration of the plate.

Summary.

1. Cases of mastitis, acute and chronic, due to pathogenic staphylococci (*Staph. aureus*) are described and the literature on the subject is reviewed.

2. In its commonest form the disease is chronic, the stroma of the organ being ultimately involved. Enduring infections lead to diffuse induration of the gland, with some decrease in milk yield and qualitative changes in the milk, such as excessive alkalinity, increase in the leucocytes, a lowered specific gravity and solids-not-fat content.

3. The importance of *Staph. aureus*, when it inhabits the udder, in part arises from the fact that without a proper bacteriological examination the disturbance it may set up may be confused with that caused by streptococci.

4. In distinguishing pathogenic staphylococci from non-pathogenic types commonly present in normal udder milk, emphasis is laid on the importance of the technique adopted. Plain agar or agar containing horse-blood should not be used. Agar containing sheep- or ox-blood is suitable, especially if the blood is taken from young animals and so contains little or no natural antitoxin, because the toxins produced by *Staph. aureus* produce characteristic changes on this medium.

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ON THE VALUE AND LIMITATIONS OF TISSUE VACCINES AGAINST RINDERPEST.

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Introduction.

Considerable work and experiment on the production and use of vaccines against rinderpest have been carried out in various parts of the world wherever this disease exists ; definite results have been obtained and its production and employment on a field scale have now been in operation over a number of years.

Although details of manufacture and formula vary in different countries and laboratories, the results obtained have been very similar in general and the subject appeared to be more or less static, or to be awaiting some improvement or alteration of technique which would increase the usefulness of the product and remove certain limitations inherent to the method.

It is convenient here to recall that such vaccines may be broadly divided into two types : one in which the tissue containing virus, finely macerated (or extracted virus as originated by Daubney), is suspended in saline, and the other in which a glycerine base is employed.

In both types the virus is inactivated or killed by some agent, formalin being probably the most generally employed, or, as in the case of original glycerine vaccine of Kakizaki, by storage at varying temperatures. The great advantage of glycerine vaccines is that they retain their antigenic potency, when stored at room temperatures, much longer than do the saline suspensions, which can only have a relatively long effective life if stored in the refrigerator. This advantage is of very considerable moment in tropical countries with difficult means of communication, since with the less permanent product, even if adequate cold storage at the producing laboratory exists, deterioration during the period of transit before injection may and probably will have occurred and its measure cannot readily be assessed.

In this Territory formalised glycerine vaccine has been in field use since 1931 and the results obtained have been satisfactory within certain limits. The main drawbacks are :

(1) Variation in the efficiency of different brews and the difficulty of adequate titration before issue of a product with a limited length of effective life.

(2) Failure to effect a " solid " immunity in animals by vaccine alone.

(3) The very definite capacity of vaccinated animals to contract Rinderpest by contact and thereon to infect other animals, although the protected animals may themselves show no appreciable symptoms on examination.

(4) The limited duration of the enhanced resistance acquired.

It is also most desirable, when having to deal with large numbers of native animals of low individual value, that the product should ensure adequate protection after a single inoculation with a dose of reasonable size. Failure in this respect may, on account of the cost of production and transport, render unpractical an otherwise useful product, and this is one reason why double or triple vaccination has only been but briefly studied at the Mpwapwa laboratory.

A Report by Bennett in 1936[1] after experiments with glycerine vaccine prepared by a method similar to that originated by Kakizaki, indicated results greatly superior to those previously obtained here. Some of his conclusions were as follows :—

> " The vaccine—virtually regardless of the dose—produces a very serviceable immunity in all cattle and a solid immunity in most cattle within three days. After a week it produces a solid immunity in all cattle.
>
> " A dose of 1 gramme per 100 kilos body weight of vaccine . . . will produce a solid immunity in a week."

In our earlier experiments we had had experience in the use of glycerine vaccines, without the addition of formalin, but had not obtained anything like such excellent results as reported by Bennett.

Accordingly, some further experiments were carried out on the efficacy of vaccine prepared by this method on native cattle in this territory.

Rinderpest is *par excellence* a disease that demands experiment and trial of the products used in its control under conditions pertaining locally. There appears to be no doubt that the inherited resistance of races and individuals varies somewhat widely from place to place. For example, in this territory we have three types of cattle of different degrees of resistance : the long-horned Ankole which is very susceptible and is subject to very heavy mortality ; Zebu cattle which now possess a useful amount of resistance to the disease, and imported European breeds and grades between these and the indigenous Zebu stock, which occupy an intermediate position but approximate closer to the Zebu than to the Ankole in this respect. The difference in the behaviour of goats to Rinderpest virus in India and Africa is another example of the same phenomenon.

Experiment I.

Results obtained with Glycerine Vaccine, incubated at 37° C.

The vaccine was prepared from portions of spleen taken from four oxen infected with Rinderpest. These were minced, shaken in the glycerine mixture with glass beads for one hour, and strained through copper wire gauze (a paint mill as used by Bennett was not available). After three days' incubation at 37° C., eight oxen were injected with doses corresponding to 1 gramme spleen per 100 kilos body weight ; there was no storage subsequent to incubation. The animals were tested ten days later by the subcutaneous injection of 10 c.c. virulent blood. No reactions to vaccination were

detectable ; temperature records were normal. The results of the virus test are shown in Table I.

Table I.

Ox No.	*Symptoms on Test.*	*Result.*
4486	Irregular temperature records up to 102°. No clinical symptoms.	Recovered ; withstood hyper-immunisation three weeks later with no reaction.
4487	Low temperature curve commencing 6th day ; peak temperature 102·6°. Definite symptoms of illness on 10th day, nasal discharge, inappetence, sick for three days, then rapidly recovered.	Recovered.
4488	No temperature reaction. No symptoms.	Recovered ; withstood hyper-immunisation three weeks later with no reaction.
4489	Doubtful temperature curve, temperature rose 6th day 101·8° ; no other symptoms.	Recovered ; withstood hyper-immunisation three weeks later with no reaction.
4490	Definite temperature reaction commencing 6th day ; peak temperature 103·4°. No symptoms.	Recovered.
4491	No temperature reaction. No symptoms.	Recovered ; withstood hyper-immunisation three weeks later with no reaction.
4492	Irregular temperature records up to 101·8°. No symptoms.	Recovered.
4493	Doubtful temperature curve ; temperature rose to 101·4° on 6th day. No symptoms.	Recovered.
4494	Irregular temperature reaction commencing 4th day. Clinical symptoms observed 10th day and the animal died on the 12th day. Blood smears showed *T. congolense* throughout the reaction and this infestation was very heavy prior to death.	*Post-mortem* : Lesions of Rinderpest of moderate severity in abomasum, caecum and colon. Death was attributable to the combination of Trypanosomiasis and Rinderpest.

Controls.—Four controls received the same infective blood as the vaccinated animals. Their temperatures commenced to rise on the fourth day and they were slaughtered for virus on the seventh day. *Post-mortem* : the usual lesions of commencing rinderpest were shown.

The results of this test were very similar to what we should have expected to occur with our routine formol glycerine vaccine, though amongst animals tested after vaccination at the laboratory appreciable symptoms and sickness have been rare. The death recorded in the table is, however, not fair evidence of want of efficacy of the vaccine as the reaction was complicated by *T. congolense*, which was in all probability a recent infestation developing simultaneously with rinderpest. The series serves to show very clearly the variability of response which vaccine engenders in the individual animal.

Experiment II.

A further series with a fresh brew of vaccine gave the results shown in Table II. The vaccine was again prepared from spleen tissue of four reacting animals and six susceptible oxen were vaccinated; there was no storage of the vaccine after incubation.

Table II.

Ox No.	*Dose Vaccine.*	*Interval before Test.*	*Symptoms on Test.*	*Result.*
4606	1 gramme spleen per 100 kilos	10 days	High temperature reaction commencing 3rd day; peak 106·2° Clinical symptoms 7th day: diarrhœa, inappetence, ulceration of buccal mucous membrane	Recovered
4608	1 gramme spleen per 100 kilos	10 days	High temperature reaction commencing 3rd day; peak 105·2°. Clinical symptoms 7th day. Reaction comparable to 4606	Recovered
4610	2 grammes spleen per 100 kilos	23 days	Definite temperature reaction commencing 4th day; peak 104·8°. No other definite clinical symptoms	Recovered
4611	2 grammes spleen per 100 kilos	23 days	Definite temperature reaction commencing 4th day; peak 105·2°. No other clinical symptoms.	Recovered
4607	1 gramme spleen per 100 kilos	—	The animal was placed in the same stall in contact with 4606 and 4608 when these animals were injected with virus. It showed no reaction. These animals were removed and 4610 and 4611 substituted. The temperature of 4607 commenced to rise 11 days later and a typical rinderpest temperature curve resulted; there were no other symptoms. The presence of virus in the blood of 4607 was demonstrated by inoculation into a susceptible ox	Recovered
4609	1 gramme spleen per 100 kilos	—	The animal was placed in contact in the same stall with routine reacting cattle inoculated for the production of virus, or vaccinated animals undergoing virus test. Freshly inoculated oxen were substituted weekly until infection occurred. Ox 4609 showed no reaction for six weeks. In the 7th week, 52 days after vaccination and 42 days after contact with infection, the temperature rose. A typical thermal reaction resulted with slight lacrymation and small buccal ulcers, but no intestinal disturbance nor lack of appetite. Actually the infecting animals were vaccinated cattle undergoing virus test (No. 4598 and 4499—Table III)	Recovered

The results obtained with this test were somewhat similar to those of the first series. It should be noted that the vaccine in doses of 2 grammes per 100 kilos body weight failed to effect a solid immunity or a blocking out of the temperature reaction.

The experiment of infection of vaccinated animals by contact will be considered in a subsequent part of this article.

Experiment III.

Comparison of Spleen Vaccine inactivated by Incubation at 37° C. for three days and similar Vaccine inactivated by Formalin 1 : 1000.

Two samples of vaccine were prepared from the spleens of three oxen, minced and suspended in 60 per cent. glycerine. One sample was incubated, the other had formalin added, 1 part per 1,000 vaccine, and was kept at room temperature for three days. Eight oxen were injected with the vaccine, four with each sample, with no additional storage of the vaccine.

The results of a subsequent virus test are shown in the Table III. All animals received vaccine at the dose of 1 gramme spleen per 100 kilos body weight unless otherwise stated.

Table III.

Ox No.	*Vaccine.*	*Interval before Test.*	*Symptoms on Test.*	*Results.*
4598	Incubated	14 days	Temperature reaction commencing 3rd day ; peak 105°. No other clinical symptoms	Recovered
4599	„	„	Temperature reaction commencing 3rd day ; peak 103·4°. No other clinical symptoms	Recovered
4600	„	„	Temperature reaction commencing 3rd day ; peak 105°. No other clinical symptoms	Recovered
4601	„	„	Temperature reaction commencing 4th day ; peak 103·6°. No other clinical symptoms	Recovered
4602	Formalised	„	Temperature reaction commencing 4th day ; peak 103·4°. No other clinical symptoms	Recovered
4603	„	„	Blocked out reaction but virus was proved present in blood on 7th day by inoculation into a susceptible ox. No clinical symptoms.	Recovered
4604	„	„	Temperature reaction 3rd day ; peak 105°. No other clinical symptoms	Recovered
4605	„	„	Temperature reaction commencing 3rd day ; peak 104·6°. No other clinical symptoms	—

TABLE III—*continued.*

Ox No.	*Vaccine.*	*Interval before Test.*	*Symptoms on Test.*	*Results.*
283 20 mths. old ¾ grade heifer	Incubated. Dose approx. 1·8 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 4th day ; peak 105·2°. No other clinical symptoms	Recovered
286 13 mths. old ¾ grade heifer	Incubated. Dose approx. 2 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 3rd day ; peak 106°. No other clinical symptoms	Recovered
296 10 mths. old ¾ grade heifer	Incubated. Dose approx. 2·6 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 4th day ; peak 105·4°. No other clinical symptoms	Recovered
301 8 mths. old ⅝ grade heifer	Incubated. Dose approx. 3·6 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 4th day ; peak 103·8°. No other clinical symptoms	Recovered
284 18 mths. old ¾ grade heifer	Formalised. Dose approx. 1·8 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 4th day ; peak 105°. No other clinical symptoms	Recovered
293 12 mths. old ⅞ grade heifer	Formalised. Dose approx. 2 grammes spleen per 100 kilos	12 days	Temperature reaction commencing 5th day ; peak 104·8°. No other clinical symptoms	Recovered
294 11 mths. old ⅞ grade heifer	Formalised. Dose approx. 2·7 grammes spleen per 100 kilos	12 days	Erratic temperature curve ; 103·4°, 5th day. No other clinical symptoms	Recovered
299 8 mths. old Pure Ayr- shire heifer	Formalised. Dose 3·6 grammes spleen per 100 kilos	12 days	Erratic temperature ; 103·6° ten days after virus inoculation. No other clinical symptoms	Recovered

The animals Nos. 283 to 299 were all three-quarters or seven-eighths Ayrshire Zebu Grade heifers. The vaccines used were the same brews as for the preceding animals but had been stored 31 days in the refrigerator.

In this series there was no appreciable difference in the protection afforded by the two samples, an uninformed Veterinarian was unable to choose one group from the other. This brew of vaccine appeared to be superior to those prepared for the series shown in Tables I and II, but neither the incubated nor the formalised sample appeared to

be as good as some samples which had been prepared here in the past and which had given a high proportion of blocked out reactions.

It is of interest, and appears to be of value, to compare the results of formalised vaccine prepared and tested at this laboratory in former years, and glycerine vaccine in which inactivation was effected by storage at room temperature. These vaccines differed from the brews prepared for the experiments described only in including a vehicle of 50 per cent. glycerine instead of 60 per cent. and in containing one part of spleen pulp to four parts glycerine solution as against one part spleen to two parts glycerine solution.

An instructive series was recorded in 1931[2]. Thirty-seven oxen were vaccinated at the same time with one brew of vaccine (one part spleen and gland to four parts 50 per cent. glycerine ; formalised 1 : 1,000), 33 received 15 c.c. (approximately 1 gramme per 100 kilos body weight) and four 10 c.c. (approximately 0·6 gramme per 100 kilos body weight). They were tested in three groups of 11, 13 and 13, after 13 days, 20 days and 27 days respectively.

The results of the virus tests were as follows :—17 showed a slightly erratic temperature never exceeding 102° C. and no clinical symptoms, 13 showed no recognisable reaction either thermal or clinical, and seven showed a typical temperature reaction with no clinical symptoms. Thirty-six of all these experimental animals showed no reaction to subsequent hyperimmunisation, but one beast which showed no reaction to virus-test (*i.e.*, a blocked out reaction) broke down to hyperimmunisation performed eight months later ; the clinical reaction was relatively mild and the animal recovered.

In this test there appeared to be no difference between a dose of 15 c.c. and 10 c.c. of vaccine but the number of animals receiving the smaller dose was, as has already been stated, only four. Incidentally no brew of vaccine has, either before or subsequently, given results superior to this series, and we have formed the opinion that such results are the best that can be hoped for with the method.

Our records of animals vaccinated with similar vaccine, but without formalin, inactivation being effected by storage in the dark at room temperature, are fewer in number. An experiment in 1930[3] can be summarised as follows :—

Of seven animals vaccinated with 15 c.c. (approximately 1 gramme per 100 kilos) and tested with virus after periods of one to three weeks, one animal contracted acute rinderpest and died, five developed thermal reactions and no other symptoms, while the remaining animal showed a thermal reaction and slight diarrhœa, *i.e.*, clinical manifestations were extremely mild and he recovered.

Examination of all the results submitted indicates that there is but little difference in the antigenic properties of the types of vaccine tried, whether they be inactivated by 1 in 1,000 formalin, three days at 37° C. or storage at room temperature.

One point we would stress is that, under the conditions in this Territory and with either native cattle or European grades, no single brew has been capable of provoking a " solid " immunity in every

animal or even in a majority of the animals vaccinated with the doses stated. That vaccine alone holds out but little promise of producing a solid immunity in all animals is indicated by our experiments with multiple vaccinations. A series of triple vaccinations in 1931[4] gave the following results :—Ten animals (two natives, two pure-bred imported European bulls and six grade ozen) were vaccinated three times at intervals of two, three, five or seven days with doses of 10 c.c. (one European bull received a 20 c.c. dose), and tested after periods ranging from 11 days to 36 weeks.

Six showed definite thermal reactions but no clinical symptoms, the remaining animals showed a slightly erratic temperature never exceeding 102° and no other clinical symptoms.

As will be shown later there is reason to believe that even a slight variation in temperature records, following inoculation of virus, may actually be a reaction, in that virus does, during this period, establish itself and multiplies in the animal's system.

The variation in response to vaccine of the individual animal has already been referred to in commenting on the series of Table I and many other similar samples are available in the literature. A conclusion of Hall's[5] that " the degree of resistance depends not only on the quantity and standard of the vaccine injected, but upon an animal's natural resistance too " appears to be well justified.

The Infectivity of Vaccinated Animals subsequently Infected with Virus.

It has been shown that animals vaccinated with formol saline vaccine[6] and glycerine vaccine[7] can contract rinderpest by contact and that vaccinated animals infected with virus are infective to susceptible animals or animals partially immunised by vaccine.

It has, however, been suggested that the risk of infection from such vaccinated animals is much less than from animals which have acquired infection naturally or which have been double inoculated and also that vaccinated animals might be likely to escape infection under natural conditions.

The contact infection experiment shown in Table II indicates that this is not the case, and the fact that ox 4609 was in contact with infection for 42 days before showing any reaction is evidence of the uncertainty of contact infection and not of resistance of the vaccinated beast. Actually a susceptible native ox (4588) had been in contact with 4607 and his infective companions for these 42 days and did not become infected. He was then tested with virus and reacted. Cooper[8] records a case in which a susceptible bovine remained under risk of infection for 33 days before succumbing to infection.

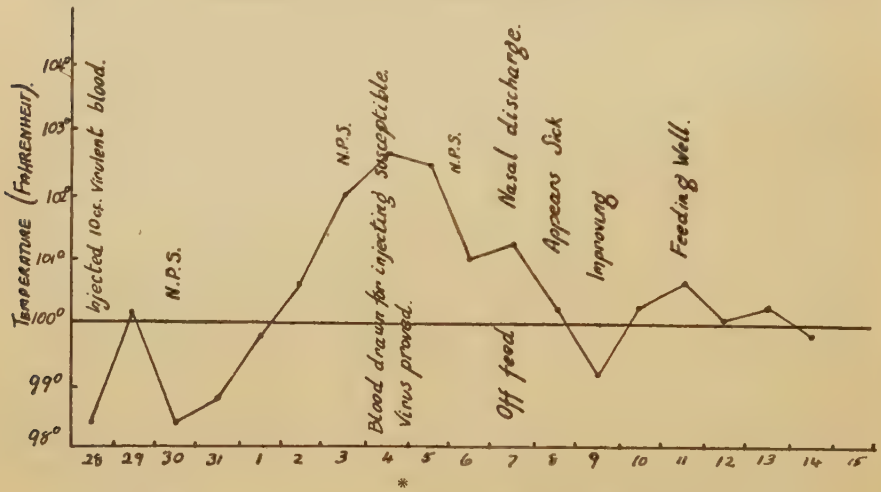
A further point of interest was noted in the case of ox 4607 (Table II). This animal acquired infection by contact and on the third day following the initial rise of temperature a blood sample was taken. This blood diluted one in 1,000 infected a susceptible ox in a dose of 10 c.c. (*i.e.*, 0·01 c.c. of blood) ; this indicates a high concentration of virus in the blood.

These facts—contact experiments carried out by Hall[6] in Nigeria, and contact experiments carried out here in 1930 with vaccinated cattle—suggest that any partially protected animal which reacts with fever to infection, is as infective as any reacting animal not so protected.

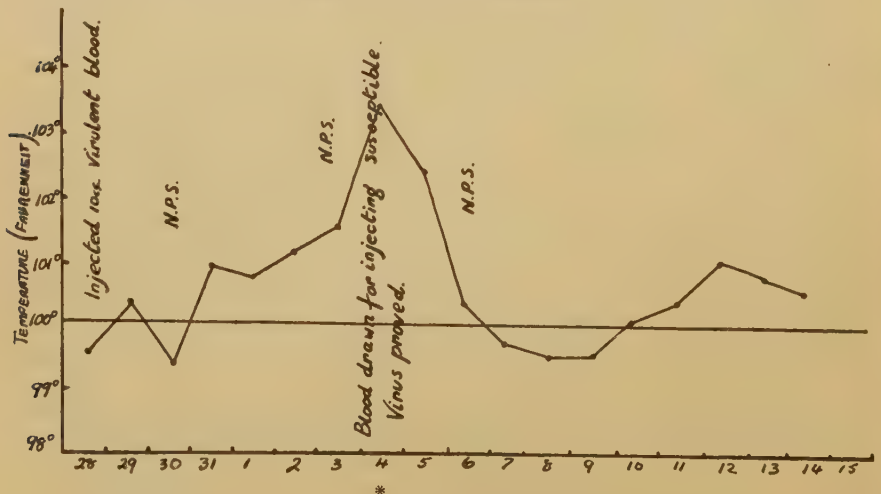
In addition we have (1930)[9] infected two vaccinated animals with 0·002 c.c. virulent blood.

THE FOLLOWING TEMPERATURE CHARTS SHOW THE DEGREE OR ABSENCE OF FEVER ON OCCASIONS WHEN THE BLOOD HAS BEEN FOUND TO CONTAIN VIRUS.

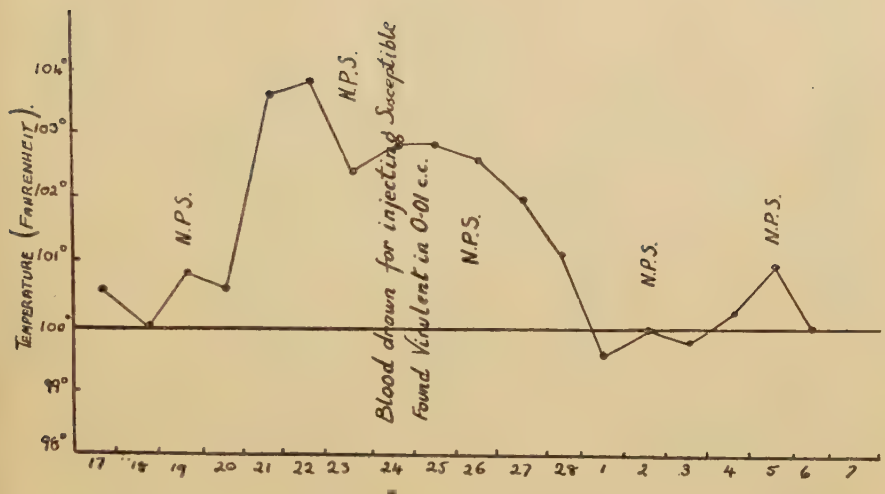
N.P.S. = No parasites seen in blood smears which were examined twice weekly.



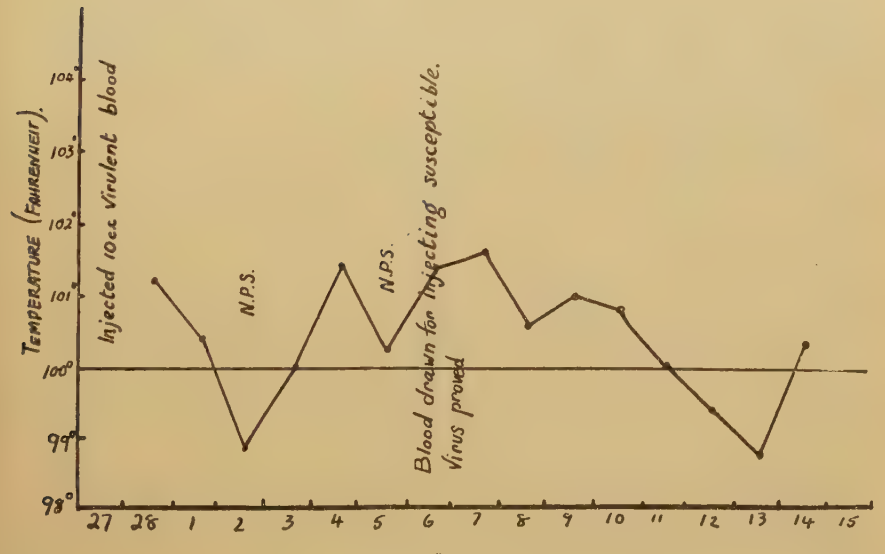
* Ox 4487. Vaccinated 1 gramme spleen per 100 kilos. Incubated vaccine. Temperature reaction and moderate clinical symptoms.



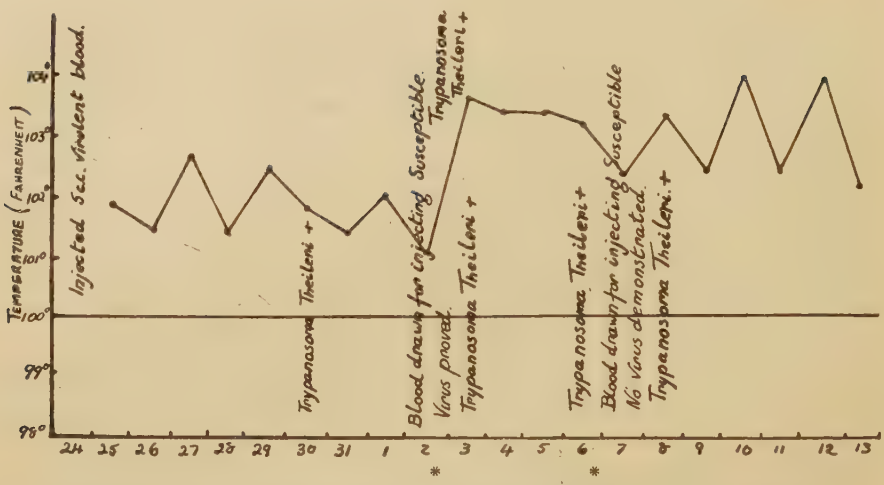
* Ox 4490. Vaccinated 1 gramme spleen per 100 kilos. Formalised vaccine. Temperature reaction, no clinical symptoms.



* Ox 4607. Vaccinated 1 gramme spleen per 100 kilos. Incubated vaccine. Infection by contact. Temperature reaction, no other clinical symptoms. Virus present in 0·01 c.c. blood on 7th day.



* Ox 4603. Vaccinated 1 gramme spleen per 100 kilos. Formalised vaccine. No temperature reaction, no clinical symptoms.


Temperature (Fahrenheit)
Injected Sec. virulent blood.
Trypanosoma Theileri +
Blood drawn for injecting Susceptible.
Virus proved.
Trypanosoma Theileri +
Trypanosoma Theileri +
Blood drawn for injecting Susceptible
No virus demonstrated.
Trypanosoma Theileri +
24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13
March, 1937.
April, 1937.

* Pure Ayrshire heifer 299. Vaccinated 3·6 grammes of spleen per 100 kilos. No clinical symptoms.

Discussion.

When a vaccinated bovine is injected with rinderpest virus it appears that any of the following sequelae may occur :—

(1) The animal develops rinderpest, the severity of which varies with the efficacy of the vaccine and the natural resistance of the animal, and it is infective throughout this reaction. In the great majority of cases this reaction is manifest only by a temperature curve characteristic of the disease ; this is the sequel which has occurred in the majority of experiments in this Territory. The animal is subsequently solidly immune.

(2) It shows no definite evidence of any reaction, either clinical or thermal, but the virus establishes itself in the animal's system and multiplies. Virus can be demonstrated in the blood and such animals are then, most probably, fully infective. The animal is subsequently solidly immune.

(3) It shows no definite evidence of any reaction and the virus does not establish itself in the animal's tissues. Subsequently it may become again susceptible to infection after a varying period.

Sequel No. 2 is illustrated by the behaviours of ox 4603 and calf No. 299 (Table III) ; it being improbable that the temperature rise of the latter recorded on the chart from the tenth to the twentieth day was attributable to the rinderpest virus : it was indeed proved to be absent in the blood on the fourteenth day.

During the normal period of rinderpest pyrexia (fourth to tenth day) no typical temperature rise was registered and at the time the blood sample was taken and proved to contain virus no indication of a reaction could be detected either by observation or the thermometer. Jacotot[10] records two similar cases : one, a calf which was

double inoculated and the other a vaccinated calf subsequently tested with virulent blood. No demonstrable deviation from health was apparent in either animal but virus in the blood was proved by inoculation. In view of these facts, we believe that those cases which, following a blocked out reaction to virus, withstand such a severe test as subsequent hyperimmunisation, had actually, at the time of the virus test, been subject to a multiplication of rinderpest virus within their tissues. The solidly immune animal appears to be able to deal with massive infection most efficiently. Thus ox 4603 (Table III) received, 18 days after virus test, 400 c.c. virulent blood intravenously and no reaction developed. Exactly one hour after the intravenous injection, a dose of 40 c.c. of this animal's blood failed to infect a susceptible animal. Again ox 4491 (Table I) received, 21 days after virus test, 1,000 c.c. virulent blood intravenously. No reaction developed. He was bled 18 hours after injection and 20 c.c. of this blood failed to infect a susceptible animal. That mere dilution of the virulent blood injected is not the explanation for the loss of virulence is shown by consideration of the volumes concerned. Presuming that the volume of blood in the living animal is about 9 to 10 litres, the dilution of virulent blood, by that of the receivers would be 1 to 25 in the case of ox 4603 and 1 to 10 in the case of ox 4491. Thus, in 40 c.c. of blood from 4603 there would be $\frac{40}{25} = 1{\cdot}6$ c.c. of the injected blood and in 20 c.c. of blood from 4491, 2 c.c.; in both cases many times the minimal infective dose of the injected virulent blood.

Sequel No. 3 is illustrated by the animals which do not react to a virus test carried out soon after vaccination, but yet later prove susceptible. Uganda (1933)[11] yields records of two such cases: two cows had been vaccinated in October and again in November; they were double inoculated in December of the same year and showed no reaction; eight months later each received 1 litre of virulent blood intravenously, with the result that both contracted rinderpest and died. A similar case at this laboratory has already been referred to in this article and it should now be generally recognised by all who have worked with this disease that a so-called "blocked out reaction," resulting from either vaccine followed by virus or the serum virus simultaneous method, cannot be relied on to produce solid and permanent immunity.

Practical Applications.

Vaccination should be regarded as a weapon for the control of the disease rather than as a promising agent for its eradication. For reducing mortality to negligible proportions, and also for preventing the marked loss of condition which the natural disease or even double inoculation generally exacts, it is most valuable.

Followed by an injection of virus, it appears to be superior to the serum and virus method, in that the reactions are milder and mortality and loss of condition far less. The collection and restraint of large numbers of cattle for a second injection is, however, a serious deterrent in many instances and, as has been indicated, the probability that some animals may not acquire permanent immunity is an additional disadvantage.

Wide experience of its use in the field has borne out the indications of its limitations suggested by laboratory studies. It is popular with many native cattle owners who have been quick to realise its efficient control of mortality and who are equally indifferent to the danger that partially protected animals may, on exposure to infection, contract and pass on the disease to the susceptible stock of a neighbour.

Where, in this Territory, mobs of cattle, in some danger of rinderpest infection have been vaccinated and not afterwards injected with virus, it has been possible, in certain cases, to detect rinderpest in some of the vaccinated individuals some months afterwards. Spread and extension of the disease from such a source has been indicated, with a reasonable degree of certainty. Endeavours to prevent the spread of the disease by vaccinating a ring of cattle around the focus of infection have been ineffective, and found to be a source of danger, owing to the difficulty or impossibility of detecting infection in such partially protected animals.

The following statements taken from the Annual Report, Veterinary Department, Straits Settlements, 1935, are pertinent: " These observations should support this opinion that unless immunity is complete, treated animals with some degree of resistance to severe or fatal infection, whether this resistance is produced artificially or is naturally acquired (as in the cases, *e.g.*, of the Indian calves) would pass unnoticed as cases of rinderpest and be responsible for spread of infection. They also demonstrate clearly that a safe period of quarantine for a batch of animals which include those having some resistance to severe or fatal infection cannot be indicated." The facts discussed and our conclusions appear to be generally recognised. Hall, in his treatise on Rinderpest Immunisation, records the variability of response depending on individual resistance, the infectivity of reacting vaccinated animals and the limitations of vaccine methods. Curasson[12], in discussing immunity following vaccination, states: " Ils ont alors, à l'abri de la resistance partielle conférée par le vaccin, fait de la peste bénigne et aquis une immunité de très longe durée." Jacotot, quoted by Curasson in his work *La Peste Bovine* states in a similar connection: " L'état de résistance ainsi produit met les animaux à l'abri de la maladie mais non de l'infection ; cette infection semble évoluer chez eux, même en l'absence de toute manifestation extérieure, sous une forme inapparente."

Nevertheless, perusal of reports and records does convey the impression that anti-rinderpest vaccines are often employed as agents

of eradication rather than of control, and without sufficient realisation of their limitations and furnishes justification for the publication of this article.

Summary and Conclusions.

(1) Experiments with incubated glycerine spleen vaccine and formalised glycerine spleen vaccine are described.

(2) No marked difference in antigenic properties of the two products was noted.

(3) Both types of vaccine failed to produce solid immunity in all animals with doses up to 3 grammes spleen per 100 kilos body weight.

(4) The infectivity of vaccinated animals when reacting to virus, and the capacity of vaccinated animals to contract rinderpest by contact are pointed out.

(5) The nature and effect of blocked out reactions are discussed and it is suggested that the difference between those blocked out reactions which result in solid and permanent immunity and those which do not, is that virus had established itself and multiplied in the animal's tissues in the former case but failed to do so in the latter. Accordingly it appears probable that some animals during the period of the so-called blocked out reaction may also be infective to others.

(6) The practical applications of these findings are considered.

Acknowledgments.

We have pleasure in expressing our thanks to Mr. H. E. Hornby, Director of Veterinary Services, for facilities to carry out the experiments, for criticism and permission to publish this article.

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[13]———. La Peste Bovine, p. 239.

A NOTE ON THE SUSCEPTIBILITY OF THE SYRIAN HAMSTER *CRICETUS AURATUS* TO *BRUCELLA ABORTUS* BANG.

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Jewish Agency Agricultural Research Station, Rehoboth, Palestine,
and
V. Ellenbogen,
Dept. of Parasitology, Hebrew University, Jerusalem.

The routine animal for work on infectious abortion of cattle has been the guinea-pig, both for infecting with cultures and for detecting the presence of the organism in milk. The disadvantages of the guinea-pig for this purpose are the variability of the results and the relatively long period up to eight weeks which may be necessary to read the final result of an inoculation experiment. Professor Adler suggested that the Syrian hamster might be a more suitable animal than the guinea-pig for this purpose and accordingly experiments were commenced with the object of testing the susceptibility of this animal to *Br. abortus* Bang. The advantages of the Syrian hamster are that it is very prolific and can be easily reared in large numbers for laboratory purposes, and it has been used for years as a routine laboratory animal for work on leishmaniasis in the department of parasitology of the Hebrew University of Jerusalem.

Material and Methods.

The following strains were used :—

(1) *Strain No.* 1.—Obtained from the department of Bacteriology, Rothschild Hospital, Jerusalem, where it has been used for many years for routine agglutination tests.

(2) *Strain " No.* 624 *B.A.B. English 8.*"—Obtained from the National Collection of type culture, Lister Institute, received 6.11.35.

(3) *Strain " N.* 1940 *Br. abortus* T."—From the National Collection of type culture, Lister Institute, received 6.11.35.

(4) A strain obtained by inoculating 5 c.c. blood into a hamster from a local cow two weeks after abortion.

The strains varied in their agglutinability, *e.g.*, the serum of an infected cow agglutinated strain 1—1 : 400, strain 2 up to 1 : 1,600, strain 3 to 1 : 1,600, strain 4 to 1 : 1,600.

Animals were infected with material from the above strains and after various intervals were anæsthetised, laparotomised, and either a fragment of spleen removed or the spleen was punctured with a fine pipette and material inoculated on glycerine agar. The animals stood the operation well, and the operation can be repeated four times at various intervals on the same animal without inconvenience.

Cultures were maintained as a routine by Huddleson's method.

Experiments.

Strain 1.

6.11.35. A two-days-old culture on glycerine agar slant was diluted with 8 c.c. saline.

6.11.35. Two hamsters inoculated intraperitoneally with 0·5 c.c. emulsion.

10.11.35. One hamster sacrificed. Cultures from blood and spleen positive.

14.12.35. Second hamster sacrificed. Cultures from spleen positive.

24.11.35. Two more hamsters inoculated as above.

26.11.35. One hamster sacrificed. Cultures from spleen positive.

5.12.35. Second hamster sacrificed. Cultures from spleen positive.

24.11.35. One guinea-pig inoculated with 1·5 c.c. emulsion prepared adding 2 c.c. saline to two-days-old culture.

14.12.35. Sacrificed. Culture from spleen negative.

4.12.35. Two guinea-pigs inoculated intraperitoneally with 1·0 c.c. emulsion.

21.12.35. One guinea-pig sacrificed. Culture from spleen and liver negative.

26.2.36. Second guinea-pig sacrificed. Cultures negative.

The infectivity of strain 1 for guinea-pigs, probably because of years maintenance on artificial media, was very low but hamsters were readily infected.

Strain 2.

Experiment 1.—27.11.35. A two-days-old culture on glycerine agar slant was diluted with 8 c.c. saline.

27.11.35. Three hamsters ♂♂ were inoculated subcutaneously with 0·5 c.c. of the above dilution.

27.11.35. Three hamsters ♀♀ were inoculated with 0·2 c.c. of the above dilution intraperitoneally.

1.1.36. One ♂ and one ♀ sacrificed. Cultures from spleen negative. One animal showed marked splenomegaly.

9.1.36. One ♂ and one ♀ sacrificed. Both showed well marked splenomegaly (spleen about twice normal). Cultures from heart's blood, spleen and liver all positive.

29.1.36. One ♂ sacrificed. Spleen normal. Cultures contaminated.

10.2.36. One ♀ sacrificed. Spleen slightly enlarged. Culture positive.

Experiment 2.—27.11.36. Culture isolated 9.1.36 from a hamster of the above series treated as above and inoculated intraperitoneally into five hamsters and five guinea-pigs in doses of 0·5 c.c.

4.12.36. Hamsters No. 1, 2 and 3 laparotomised. All showed splenomegaly particularly hamster No. 1, in which the spleen was almost three times normal. Cultures from spleen and liver positive.

7.12.36. Four guinea-pigs died (? Pasteurella infection).

9.12.36. Hamsters No. 4 and 5 laparotomised. Splenomegaly and cultures from liver and spleen positive.

15.12.36. One guinea-pig laparotomised. No splenomegaly. Cultures from liver and spleen negative.

28.12.36. Hamster No. 1 laparotomised. Spleen normal in size and adherent to neighbouring tissue (probably as result of previous laparotomy). Culture of spleen and liver positive.

29.12.36. Hamster No. 2 laparotomised. Cultures from liver negative.
29.12.36. Hamster No. 3 laparotomised. Splenomegaly. Cultures from spleen positive.
29.12.36. Hamster No. 4 died. Spleen normal. Cultures of spleen, liver, kidneys and heart's blood positive.
29.12.36. Hamster No. 5 laparotomised. Splenomegaly very marked. Liver enlarged. Cultures positive.
30.12.36. Hamster No. 2 moribund and sacrificed. Showed peritonitis. Culture contaminated.
6.1.37. Guinea-pig died. *P.M.*: broncho-pneumonia. Spleen normal. Cultures positive.
7.1.37. Hamster No. 5 died. *P.M.*: broncho-pneumonia. Splenomegaly. Cultures negative.
13.1.37. Hamster No. 1 laparotomised. Spleen not enlarged, adherent to neighbouring tissue. Cultures from spleen and liver negative.
14.2.37. Hamster No. 1 sacrificed. Cultures from all organs negative.

Strain 3.

27.11.36. Five hamsters and five guinea-pigs inoculated with 0·5 c.c. diluted culture 1, strain No. 2, as in the previous experiments.
1.12.36. Three guinea-pigs and two hamsters died (?? of Pasteurella infection).
4.12.36. Hamster No. 3 laparotomised. Splenomegaly. Cultures from liver and spleen positive.
7.12.36. Hamster No. 4 laparotomised. Splenomegaly. Cultures from liver and spleen positive.
7.12.36. Hamster No. 5 laparotomised. Slight splenomegaly. Cultures from spleen positive.
12.12.36. Guinea-pig No. 4 died, found putrefied.
15.12.36. Guinea-pig laparotomised. Spleen normal. Cultures from liver and spleen negative.
28.12.36. Hamster No. 4 died. *Br. abortus* not isolated.
10.2.37. Guinea-pig sacrificed. Culture negative. Spleen normal. Serum gave no agglutination.

Strain 4.

27.11.36. Five hamsters inoculated as above.
29.12.36. Two hamsters died and found putrefied.
7.12.36. Hamster No. 3 died. Splenomegaly. Cultures from liver positive.
7.12.36. Hamster No. 4 laparotomised. Splenomegaly. Cultures from liver and spleen positive.
7.12.36. Hamster No. 5 laparotomised. Splenomegaly very marked (three times normal). Cultures from spleen and liver positive.
Experiments with Material from Cattle (these were limited because of an epidemic among the hamsters).
27.12.35. 3 c.c. defibrinated blood from a cow 12 days after abortion inoculated intraperitoneally into a hamster ♂.
27.12.35. 5 c.c. milk inoculated intraperitoneally into two hamsters ♀♀.
1.1.36. Hamster ♂ sacrificed. Slight splenomegaly. Cultures from liver positive. Cultures from spleen negative.
1.1.36. One ♀ sacrificed. Milk in peritoneum caseated. Cultures negative.

9.1.36. Second hamster ♀ sacrificed. Cultures from liver and spleen negative.

26.1.36. Three hamsters (Nos. 1-3) ♂♂ inoculated intraperitoneally with 5 c.c. defibrinated blood from above cow.

26.1.36. Nos. 4-6—Three ♀♀ inoculated intraperitoneally with 5 c.c. milk (cream and centrifugate).

26.1.36. Nos. 7-8—Two ♂♂ inoculated intraperitoneally with mucus from the vagina.

10.2.36. Hamster No. 7 died and was found putrefied.

13.2.36. No. 1 splenectomised. Spleen normal. Culture from spleen positive, liver negative.

13.2.36. No. 4 laparotomised, spleen normal. Cultures from liver and spleen negative.

23.2.36. Nos. 5 and 6 laparotomised. Spleens normal. Cultures positive.

23.2.36. No. 8 laparotomised. No splenomegaly. Cultures positive.

23.2.36. No. 1 again laparotomised. Spleen normal. Cultures from spleen and liver positive.

23.2.36. No. 4 laparotomised. Cultures from spleen positive, liver negative.

5.3.36. No. 5 laparotomised. Spleen normal. Cultures negative.

5.3.36. No. 2 laparotomised. Slight splenomegaly. Cultures from spleen positive, liver negative.

15.3.36. No. 2 died of peritonitis. Cultures from liver positive, spleen negative.

24.3.36. No. 1 again laparatomised. Spleen normal. Cultures from spleen positive.

20.12.36. A hamster inoculated subcutaneously 5 c.c. defibrinated blood from the same cow.

29.12.36. Laparotomy. Spleen enlarged. Cultures from liver and spleen negative.

13.1.37. Laparotomy. Cultures negative.

9.3.37. Sacrificed. Cultures negative.

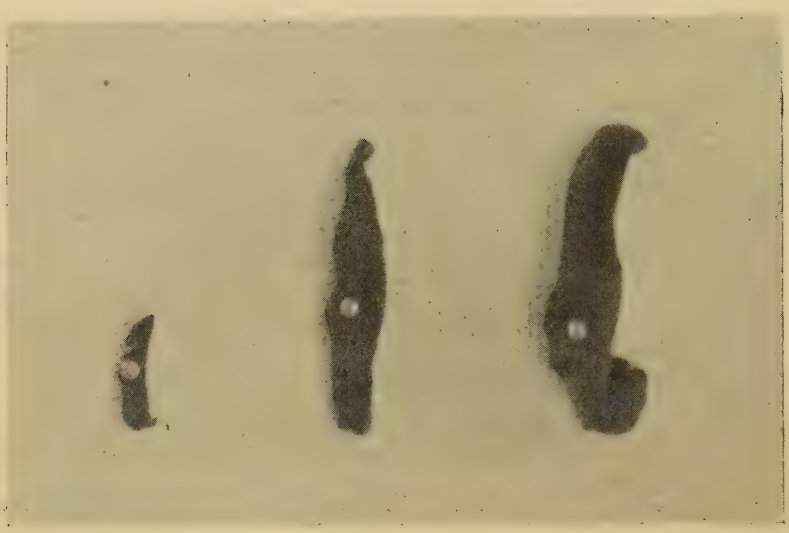
Seven other cows whose serum agglutinated *Br. abortus* were examined by inoculation of defibrinated blood and milk into hamsters. *Br. abortus* was not recovered from any of these hamsters although some of them showed a marked splenomegaly after the inoculation.

Out of a total of 21 hamsters inoculated with cultures, *Br. abortus* was recovered from 18; two animals sacrificed four days after inoculation were negative, in one the cultures were contaminated.

Out of ten hamsters inoculated with blood, vaginal mucus, milk of a cow within six weeks after abortion, seven gave cultures and three were negative. Of the latter one had peritonitis due to a contaminating organism. A single hamster inoculated from the blood of the same cow twelve months after abortion gave a negative result.

The animals were examined from four days or more after the inoculation and cultures were obtained as early as four days after inoculation with culture or with blood from an infected cow.

The degree of splenomegaly in some of the infected hamsters was very considerable, as the following photograph shows; splenomegaly



1 2 3

FIG. 1. Spleen of Normal Hamster (the length of the normal spleen varies and may be twice as much as the specimen photographed).

FIGS. 2 and 3. Spleens of Hamsters infected with *Br. abortus*. Scale indicates size in millimetres.

was in some cases already marked as early as seven days after inoculation, but there were several animals from which cultures were obtained and in which the spleen was normal in size. In one instance both the splenomegaly and the infection in the hamster appeared to be transient.

The above observations, although limited in number and of a preliminary character, indicate that the Syrian hamster is a very suitable animal for investigations on infectious abortion of cattle.

We have to thank Mr. David Stern of the Department of Agriculture of the Jewish Agency for his kindly interest in this work, and Professor S. Adler for his encouragement.

ABSTRACTS.

INVESTIGATIONS REGARDING THE INFECTION OF THE UDDER IN CONTAGIOUS ABORTION: THE CAUSE OF THE EXCRETION OF THE *BR. ABORTUS* AND ITS RECOGNITION.*

By W. Stockmayer.

At the outset the author observes that the question of the role of the udder in contagious abortion of the cow becomes increasingly important. Cows in which the udder is affected excrete the causal organisms with the milk and thereby create a centre of infection for the whole herd. The excretion is also important from the point of view of public health. The necessary measures against the excretion can only be undertaken with success when the nature of the infection in the udder has been accurately determined. When, for example, one endeavours by means of guinea-pig inoculation to discover the excretors, one proceeds on the assumption that the excretion is uniform, but how far that is correct has not been fully investigated. The author in this connection commends the investigations carried out by Dr. Zeller. It was that author who, in Germany, first (1922) recommended the addition of gentian violet to the culture medium in working with materials contaminated with abortion bacilli with the object of restraining the growth of Gram-positive bacteria. Huddleson, in 1922, and in 1927 with Hasley and Torrey, used gentian violet in combination with liver agar in the examination of milk samples taken with sterile precautions. (Very full References to other investigations are given).

The Author's own Investigations.—In the literature on the subject, it appears that in most of the investigations regarding the excretion of Bang bacilli only a small number of animals have been used from the diagnostic point of view. The necessary knowledge regarding the course and the nature of the excretion or of the infection can, however, be obtained only by systematic examinations of single animals, as shown (1932) by O. Bang and Bendixen. Investigations by the present author (1933) had already shown how much the excretion of the organisms depends upon the stage of lactation and revealed what was the time when the examination was of most importance.

The present investigations were carried out on a total of 39 cows which, in the course of another experiment concerning the estimation of the value of Bang vaccine, were infected with Bang bacteria. Concerning that experiment, Zeller and Stockmayer had already reported briefly in 1935. For inoculation only vaccine composed of killed Bang bacteria was used and it was, therefore not to be assumed *a priori* that the excretion of living Bang bacteria took place through the milk. The observations in question also gave no criterion in this respect, so the inoculations might be overlooked. On the contrary, the action of the inoculations on the agglutination value of the milk serum must be taken into consideration. The last vaccination, however, had been made from five to eight months before the first milk agglutination.

All the experimental animals were pregnant heifers which before purchase were tested with negative result. The infections were carried out in the artificial way, but as far as possible under natural conditions: cutaneous inoculation on the unwounded feet or the conjunctiva. A first

*From *Zeitschrift für Infectionskrank, Parasitäire Krank, und Hygiene der Haustier*, 1936, Band xlix, Heft 1.

series of eight animals were infected in the natural way. The course of the outbreak was acute ; in the first calving period the pregnancy of 68·7 per cent. and in the second pregnancy of 18 per cent. of the whole lot of animals ended prematurely. The agglutination titre of the blood serum was very high, in most of the cows between 1,000 and 50,000 and only occasionally below that. One animal had apparently not been infected.

This experiment created an opportunity to examine the milk of the cows throughout two lactation periods. A cultural test followed daily at the intervals chosen or otherwise in regular longer intervals. Thereby it was discovered which cows were continuously or temporarily excreting Bang bacteria with the milk and, therefore, in which of them the udder was infected. The information thus obtained formed the basis for estimating the value of milk-serum agglutination, which in the second year of the experiments was carried out at weekly or monthly intervals. For estimating the value of the culture method guinea-pig experiments were used for several months for comparison, and also the question of methods of enriching by culture methods was tested.

Cultural Investigations.—Of each cow, from the day of parturition, single milk samples were as far as possible examined daily until the cause of the excretion appeared to be essentially cleared up. This lasted at least four weeks and in a great many of the animals longer, even up to five or seven months. Later the examinations were made at monthly intervals and also repeated during the dry period.

The examination of the milk samples was carried out on gentian violet-malachite green agar plates. Only a single sample of milk was used and that was taken at the end of the milking. The nutrient medium used was Huddleson's liver agar, and later beef agar with the addition of 10 per cent. of ox serum and 2 per cent. glycerine. Gentian violet was used (Grübler, Leipzig) in a dilution of 1 : 250,000, malachite green in 500,000. The stain was kept ready for two months in a 0·1 per cent. watery solution. The pH. value was from 7·4 to 7·6 according to Michaelis.

The running examinations served to show the average number of bacteria. Care was, therefore, always taken that the samples were thoroughly shaken before the test. This was done either with the hand or with a shaking machine for at least a minute. Of each milk sample 0·05 c.c. was used for each plate, uniformly mixed with a glass spatula and then rubbed on the surface of the medium. The plates were so well dried that after inoculation they no longer appeared moist.

The experimental herd was outside Berlin and, therefore, the milk samples were brought to the laboratory twice in each week. To prevent any increase in the number of bacteria in the milk sample between the day it was taken and the day on which it was examined, 0·5 to 1 per cent. boracic acid was used. Stockmayer had previously reported regarding this (1935) and the experiments in question showed that the addition of the boracic acid restrained the multiplication of the bacteria only for four days. During the warmer period of the year a 1 per cent. solution should be used.

The investigations in question showed that the excretion varied greatly with different cows. The excretion of Bang bacteria by the udder generally begins with the period of lactation. In the stronger excretors it reached its height always in the first days of lactation. After that the number of the organisms increased gradually up to the end of the second week. This, however, did not continue until the excretion came to an end ; the bacteria only gradually became fewer, but in the continued examination they were

still present on different days but in smaller numbers. There were all conceivable stages in the interval. The excretion could be followed nearly continuously for six months ; or it might temporarily disappear and then suddenly reappear.

Brucella bacteria were also sometimes found during the first two weeks of lactation. In one cow the excretion began in the second lactation period immediately after parturition. In another case a regular excretion began first two months after parturition.

As shown in a table, the number of bacilli in the milk may be astonishingly large at the beginning of lactation, from 10,000 to 200,000 per c.c. In the further course the excretion of 2,000 per c.c. is seldom reached ; generally the number of bacteria diminished down to single organisms. It is to be noted, however, that these are the lowest numbers. There are undoubtedly more bacteria present than can be detected by culture.

On account of other defects in the method employed the numbers must be taken as only an approximate picture of the actual circumstances.

Of importance were the discovered cases of cows which apparently had no infection of the udder or not in an open and thereby recognisable form. These were subjected to a specially long daily examination in order to discover whether in the course of time occasional bacteria would not be detected. Here, again, one might suppose that the cultural methods employed did not afford sufficient assurance that very slight numbers of brucella bacteria would be detected. (This to be considered later.)

From the comparative investigations the guinea-pig inoculation was the most sensitive test, but in general the guinea-pig experiment also proved negative if during a long number of days no Bang bacteria could be obtained by culture.

By the culture method, it was possible in nearly all the animals to discover bacteria on single days if the examination was continued long enough. In one cow in 95 examinations it was possible to discover two Bang colonies. In another cow the milk after the first parturition was subjected to uninterrupted examination and the culture was first positive on the 71st day, and after the second parturition rather more frequently. In a further seven cows, the cultural examination of the milk samples was almost always positive, only in one case completely negative, and in that the guinea-pig experiment still gave a positive result. These animals were with one exception under observation in two periods of lactation and in this long interval the Bang bacteria were detected in all of them at least on occasional days.

The later examinations showed that with these occasional excretors the bacilli were rather more frequently detected by animal experiment, but that thereby the usual picture was not essentially changed.

These investigations, which were confined to animals that gave a positive reaction to a blood serological test, were completed by examination of cows of the herd which apparently had not been infected, that is to say, animals that were negative to an agglutination and a complement test. The cow after giving birth to a healthy calf was only a short time in the herd and its milk was examined only on the 16th day. On the 28th day after parturition, however, an increased number of bacteria were detected in the milk. That shows that even in cows reacting negatively occasional Bang bacteria are excreted by the udder. Unfortunately the author had no further opportunity to examine non-infected animals in this direction.

These cultural investigations show that the cows in an infected herd cannot, in regard to udder infection, be separated into excretors and non-

excretors; the cows which more or less constantly passed Bang bacteria with the milk had rather to be regarded as " permanent excretors "; and in opposition to these stand the occasional excretors, in whose milk only occasional organisms are to be found. On practical grounds the term " permanent excretor " was very widely interpreted and it included not only the animals in whose milk the bacteria were to be found during the whole lactation, but also all those which for a short period were regular excretors.

CONCLUSIONS.

1. Cows affected with brucellosis of the udder temporarily or permanently excrete the bacteria with the milk in a repeated rotation.

2. According to the author's observations the excretion began as well after the first as after the second parturition, mostly with the onset of lactation or in the following days. The number of bacteria present was also largest at this time.

3. The degree and duration of the excretion varied very much in different cows. As many strong excretors were observed in cases of normal parturition as after abortions.

4. It is impossible to define a normal duration of the excretion. In the milk samples of three cows brucella bacteria were daily obtained by culture, with short periods of interruption, during six months; in another series of cows the same result was obtained for several months. In general the number of the bacteria excreted with the milk diminished during the lactation period, but in this decline there were extensive variations. When the number of organisms present in the milk was very small they were seldom detected in the periodical examinations. In the dry period the number of bacteria in the udder increased.

5. In permanent excretors the blood serum and milk serum generally had a high agglutination titre. In the preparation of the milk serum the separation of the fat by chloroform was approved.

6. Even in cows in which the agglutination titre of the milk serum was low or negative, brucella bacteria could be detected in the milk samples on some days (occasional excretors).

THE AGGLUTINATION TEST FOR THE DIAGNOSIS OF EPIZOOTIC ABORTION.*

By DR. STABLEFORTH.

THE author's principal object was to compare the suspensions, methods and criteria of diagnosis used by different experimenters in different countries in the practice of the agglutination test for the eradication of epizootic abortion. Some details regarding observations made in a herd are intended to accentuate the fact that the sero-agglutination test may sometimes fail to reveal a considerable number of infected animals when it is made immediately after abortion or normal parturition.

The agglutination test is undoubtedly the one which is most extensively used for the *Br. abortus* and it constitutes the basis of all the systems of eradication. Nevertheless, there is a great divergence in the new criteria

*From article by Dr. Stableforth in Tome XII, International Office of Epizootics, p. 107.

adopted in different countries as indicators of infection or probable existence of infection. This divergence may have two reasons; the first is that the methods and the suspensions used for the test are different, or secondly, that the criteria are in reality more severe in one case than in another.

In order that the discussion between experimenters in the same or in different countries may be profitable, it is essential that the titre obtained by a given serum with diverse suspensions and methods shall be the same or that the rapports of the suspensions and methods may be known.

(The author then proceeds to give what he describes as a short sketch of the methods adopted in this country.)

It is stated that the suspension which is generally adopted in Great Britain for the diagnosis of epizootic abortion is prepared under the auspices of the Agricultural Research Council (Great Britain) and it is furnished to other laboratories with a programme implying collaboration in the prevention of abortion. It is a suspension of uniform agglutinability, standardised by titration of a series of exact dilutions of a dry, stable, agglutinating anti-abortion serum. The desiccated serum has retained its agglutinating power without change during the three years from the date of its preparation, and, after observations made during 10 or 15 years on other anti-serums, it may be expected that its power will be maintained almost indefinitely. The opacity of the original suspension is about that of tube 4 of Brown's standard of opacity with sulphate of barium (Brown and Kirwan, 1915)—a standard of which the opacity is about twice that obtained by the mixture of 300 cubes of barium chloride at 1 per cent. and of 97 c.c. sulphuric acid at 1 per cent. The opacity of the suspension is a little different because the uniform agglutinability is realised by slight modifications of this opacity to the final point desired, corresponding to an agglutination of 25 per cent. obtained with a dilution of 1 per 480 of desiccated serum; one compensates in this way the differences which exist in the natural agglutinability of each suspension. The opacity of the mixed serum-suspension is then approximately that of Brown's tube 2.

The author states that it is easy to understand that any method of diagnosis may fail when the test is made immediately after infection. Moreover, when it is a case of a method that is indirect, such as the agglutination test, one may expect that the interval between the actual infection and the appearance of antibodies in the circulation may be considerable since it depends on the dose, the port of entry, and the susceptibility of the animal. Hence it is not surprising that an animal may be negative to a test made at the moment of purchase and that it may be positive some weeks or months later. It is, however, not so easy to understand why an animal fails to react in a series of tests covering a period of 12 months or more in an apparently non-infected environment and then quite suddenly reacts. When such a case occurs, not before but after parturition or abortion, the result may be a new propagation of the disease. One has known, however, for some time that facts may occur in that order, but nevertheless the literature on the subject gives the general impression that such a case is not usual, and that the percentage of infected animals which react only after parturition does not exceed 5 to 10 per cent. Unhappily there is accumulating evidence that cases of this nature are more frequent than is generally supposed, and that in certain herds they may prove a serious obstacle to a programme of eradication. The author observes that in this connection the observations made by Karsten and by Hayes and Barger (1935) are important.

The paper then proceeds as follows :—

Karsten (1936) gives details regarding a herd of 240 adult animals, in which since March 1931 material taken from fœtal membranes of each animal after normal parturition (673 in number) has been inoculated to one or two guinea-pigs. In 33 cases *Br. abortus* was isolated although the cows had not reacted either before parturition or at the moment of parturition, and in 21 cases they had remained negative up to the date of the report. The blood and the milk had been tested.

Br. abortus had also been similarly isolated from the milk of three cows which had never reacted. Six other cows calved before reacting. It is said that probably the method of test employed was the official German one and it is pointed out (by *Stableforth*) that the criteria and the methods had only revealed reactions of a low titre and that in consequence some of the animals may have reacted at this moment with a low titre.

Hayes and Barger (1935) also indicate that certain cows may excrete *Br. abortus* several months before agglutinins can be detected in the blood serum ; that some cows which, according to their history have given positive blood reactions, may excrete *Br. abortus* some time after their titre has become negative (1 : 100 with their test, that is, 1 : 10 with the authors) and that some cows of which the history reveals constantly negative tests over a long period may occasionally be carriers of the *Br. abortus* and excrete that organism at the time of a normal parturition or more frequently at the time of an abortion. Haring and Traum (1936) cite similar interesting observations on the same subject.

In the case of Hayes and Barger, and the herd tested by the author, the question is not one of those which are notoriously influenced by the relative titre considered to be proof of infection, since the great majority of the animals did not show any trace of reaction, even to the author's 1 : 10 dilution, until after parturition or abortion. From a practical point of view antibodies in the circulation did not exist.

The following is the description of a herd said to be typical of many herds of the same kind known to the author.

It included about 50 head in October, 1923, 49 per cent. of them being reactors. The reactors and the non-reactors were separated completely, and the buildings and the management were adapted to the application of a plan of eradication. Many abortions had occurred ; they continued among the reactors in the first year of the plan. Nevertheless, the number of the reactors gradually diminished. Only five were found at the third, fourth, fifth and sixth tests or between these tests. None of these cows had recently calved, and the milkers regularly washed their hands after the milking of one cow before proceeding to the next, so that a spread of the disease from this source appeared improbable. All the reactors were put out of the herd immediately.

In May, 1935, none of them remained at the farm and the buildings which had been utilised for the reacting section of the herd were thoroughly disinfected.

The seventh test of the herd, in July, 1935, included all the animals ; and no reaction occurred in any of them, even in a dilution of 1 : 10.

In July, 1935, a cow which had been purchased ten months previously and which had been submitted to four tests without giving a positive reaction, aborted a seven-months' fœtus. The event was discovered in time and the animal was transported to a box. *Br. abortus* was isolated from the fœtus. A table in the article shows that since this case of abortion five cows had

aborted in succession (caused by *Br. abortus*) and that seven others had been infected (three of which calved at the moment or almost at the moment of the test), a total of twelve distinct infections. Four other cows had aborted or calved prematurely, but none of them had ever reacted (one had three tests since the abortion) and *Br. abortus* was not isolated from the fœtus of any of them. Eleven of the twelve infected cows had been purchased from six months to a year previously, and all with the exception of one showed the infection during or at the end of their second period of pregnancy.

The most important point was that five of these animals gave negative results to the last agglutination test before the act of abortion or of parturition, respectively one week, five weeks, two weeks, two-and-a-half months, all entirely negative. Four others which had been equally negative two months previously were eliminated because on December 4th they gave positive reactions, respectively at 1 : 20 ; 1 : 160 ; 1 : 10, and 1 : 20. Two of these cows aborted, one of them after two days and the other after five, and two calved at full term. The three other infected cows have not yet aborted or calved. The five animals which gave negative reactions before parturition reacted at 1 : 10, 1 : 20 one or two days afterwards, and 1 : 40 after two to three weeks.

" It is difficult to be sure that some of these animals, or even the whole of them, had not been recently infected. One or several of these animals which had constantly given negative reactions may at some moments have excreted *Br. abortus*. Nevertheless, the tests as a whole are against such a probability ; and, in the first place, the precautions taken to avoid a possible spread of the infection, even in the herd giving negative reactions, were appropriate ; and secondly, the animals from the moment of purchase, six to twelve months previously, had been kept in the same stable and fed and pastured together with a nearly equal number of animals of the same age born in the herd. None of these animals became infected. If, as appeared possible, they had been carriers of the infection for six to twelve months, the fact that a large number of them, five out of twelve, have not reacted until after parturition or abortion is of considerable importance. Three others reacted to a test at a very low titre in December, 1935 ; they would have passed undetected if the test had been carried out some weeks sooner although two aborted respectively two and five days after the test of the herd carried out in December. They would have aborted in the herd giving negative reactions if the test had been made a week later or if the criteria of infection had been a little less severe."

The author found it impossible to give the details regarding the other herds, but a succession of somewhat similar facts to those recorded in this herd occurred in three others with which the author had been concerned, and he adds that it was known that similar facts had been observed in other herds in Great Britain.

" The number of herds in which the eradication plan has not given favourable results is not great ; in the majority of the cases the results have been eminently satisfactory, but the failures when they occur are serious for the owner and it is probable that they delay the normal expansion of the plan. The question which is posed by the author is whether some other test, such as fixation of the complement or an intradermic test has any value for the detection of animals which although infected do not react to the agglutination test."

The author observes that in undertaking this work he knew that the criteria adopted for the agglutination test in the diagnosis of contagious

abortion in cattle varied in different countries. There were several technical reasons that might explain this diversity. In the first place, it was known that suspensions differing in opacity, that is, in their richness in bacteria, would agglutinate in different degrees in the presence of the same serum ; secondly, that suspensions of the same opacity, but prepared with different strains or even with the same strain, could vary in respect of agglutinability, and thirdly, that possibly a difference in the method of carrying out the test might influence the result obtained with a given serum and suspension. It has been demonstrated that one of these three technical factors, or all three of them, exercise their influence in the diverse methods examined, among which no two were found exactly parallel. It was known that after having taken account of the differences in the methods there still existed a great divergence in the real severity of the criteria of diagnosis utilised in the different countries.

The eight different methods which have been compared have been adopted in the majority of cases for the purpose of a comprehensive plan of eradication, official or unofficial. In the other cases the central laboratories carried out a considerable number of tests with a view to the control of the disease. " The bases of comparison have naturally been our own method and our criteria, in which we used standard suspensions with a uniform agglutinability obtained with certainty by titration with a dried, stable, anti-abortus, agglutinating serum."*

" The great difference in the richness of the suspensions in bacteria used in the different methods described is surprising, even if one takes into consideration the fact that in some of these methods the serum is directly diluted in the suspension or the entire serum is added, whereas in other methods the dilutions of serum are first made in a saline solution and an equal volume of suspension is then added. The proportion of bacteria present in some suspensions is ten times that of others. It is this difference which is principally responsible for the different titres given by different tests. One factor intervenes—the coincidence that the three suspensions in which the proportion of bacteria was the feeblest also contained the bacteria that were most sensitive. This difference in sensitivity of the bacteria considered *per se*, contained in the suspension, but was not always greater than that which one could expect in it, some having a sensitivity about twice that of another, and the difference was a little greater than what one observes in suspensions prepared in the same laboratory for the utilisation of the same methods and the same strains. Some suspensions, notably the suspension D., gave a final point more clearly than, for example, suspension H., and it is interesting to note that the suspension D. had been prepared with bacteria which had been centrifuged and washed three times. The suspension H. would have been improved by a more appropriate filterage."

" As has been demonstrated even when the suspensions used are of a similar agglutinability, the differences in the method in which the test is carried out may have a great influence on the signification that is to be given to a nominal titre. Although the nominal titre may be the same in two methods the real relationship of the quantities of serum and bacteria may in the one case be double that of the other case. For the profitable discussion of the signification of the agglutination to a given titre it is necessary to know the details of the method or, more simply, the opacity of the mixed serum-

*For the details of the preparation of the dry serum, see this Journal, vol. xlix, p. 251.

suspension. This opacity in the first place affords information on the relative proportions of bacteria and serum present in a given dilution, independently of the method or of the original suspension used."

" There remains a variable factor, the sensitivity of the bacteria themselves. Usually in a laboratory it is measured by testing each suspension to a given opacity with a serum reserved for this purpose. However, if one desires to measure the agglutinability of suspensions prepared at long intervals it is desirable to have the certainty that the standard serum retains its proportion of antibody unchanged for as long a time as possible. For this purpose a desiccated serum such as we have adopted and found satisfactory is clearly the best because the desiccated sera retain their titre in antibody almost indefinitely."

" The slight reduction in the titre caused by heating to 50° C. for 40 minutes applies equally to sera of feeble and high titre, and it seems that heating has no advantage. The inconvenience of heating, that is to say the production and intensification of zones of inhibition which we have mentioned, agrees with the previous observations (Spencer, 1930; Priestley, 1931), and with the fact that the incubation of tests at 55° C. provokes or intensifies such zones."

Note by the Editor.—Dr. Stableforth appears to have been moved to write this article because he failed to use the agglutination test with success in an endeavour to eradicate abortion from a particular herd. It was obviously a milking herd and the fact that he expected success and still thinks he might have won it is part of the evidence that he had not yet given serious attention to the history of the disease.

Well-informed readers will pay no attention to the part which describes his manipulations with the suspensions of abortion bacilli which he obtained by request from five different countries for comparison with his own. They will also ignore the suggestion that desiccated serum is necessary in the agglutination test. That borders on the nonsensical.

The serious part of the paper is that devoted to showing that the agglutination test is not trustworthy and that a search for a better one ought to be made. But that is likely to be serious only to the reputation of the author.

What may be called the core of the paper is the author's personal contribution to this question in an account of a herd in which the agglutination test failed in his own hands. A strange thing about this case is that the author has maintained silence regarding it for 14 years. It is impossible to see in it any reason for breaking silence now, as it clearly was a herd in which an attempt to eradicate the disease ought not to have been made. Warnings had already been published against such an attempt in the circumstances described.

The author leaves it to be understood that the agglutination tests in this herd were carried out by him at the Research Institute, Royal Veterinary College, but such was not the case. The test in question was carried out without the knowledge or consent of the Institute, and the opinions which he now expresses would have been repudiated as entirely contrary to the results of the very extensive researches carried out at the Institute and published during the previous twelve years.

It is not out of place to state that at the date of this test (1923) the author was 21, and he had only recently been admitted a member of the Royal College of Veterinary Surgeons. The account which he now publishes implies that no one was associated with him in his researches at that time (or since).

It is a great fault in Dr. Stableforth's first contribution to the literature on contagious abortion that where published researches are not in agreement with his own conclusions he ignores them. This is a charge that he can escape only by admitting that he has not taken the least trouble to discover by reading whether his present opinions regarding the agglutination test are new or old, confirmed or obsolete.

Take for example his opinions on the critical titre of agglutinin in the test. Common decency demanded that in laying down the law on this subject he should have stated why he disagreed with the opinion generally held, that 1 : 10 is the titre of normal bovine animals.

If he wishes to retain a reputation for candour he must now admit either (1) that he was ignorant of the fact that Henricsson found that in the far north of Sweden (Lapland), where no case of contagious abortion had been known for 50 years, the blood serum of the native cows failed to cause agglutination at 1 : 10, or

(2) that he suppressed this knowledge because it was fatal to his main contention. And he is in the same dilemma in regard to the numerous articles previously published in this Journal, in which proof was provided that to ignore a reaction at 1 : 10 in testing the blood of cattle in England allowed some infected animals to escape detection and was thus an obstacle to eradication of the disease from a herd.

We prefer to think that the explanation of the author's lack of knowledge on this subject may be due to a vow that he would not read any article on the agglutination test if it ran counter to his own preconceived ideas on the subject.

The author's logic appears to be as weak as his knowledge regarding the agglutination test. It will be observed that he claims that the agglutination test has been generally successful in his hands, although nearly the whole of his paper is devoted to showing that it ought to be discarded. In making this statement he appears to have been blind to the fact that when he found the test generally correct he ought to have ascribed its occasional failures to some error in his manner of employing it.

He would do well now to reflect that others, with one hundred times his opportunities for studying the question, have published facts completely inconsistent with his opinions regarding the agglutination test, and to consider the possibility that he is mistaken. Or has he reached the " fact proof " stage of conviction ?

In publishing an account of this herd in evidence of the failure of the agglutination test he certainly has shown the courage of his convictions. It is not everyone who would have risked his professional reputation by giving publicity to the fact that he did not think it necessary to have separate milkers for the diseased and the healthy sections of the herd provided they washed their hands after milking.

Perhaps the herd was his own, and in a sense that would have been very appropriate, but he ought to have remembered the adage about the lawyer who makes his own will.

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